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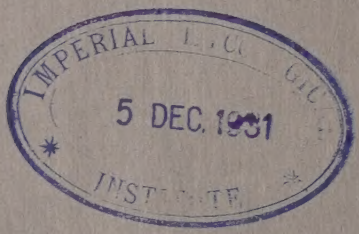

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The Diagnosis of Species of Fusarium by Use of Growth-Inhibiting Substances in the Culture Medium

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In preliminary publications (1, 2), the situation which exists in the genus Fusarium has been sketched and the difficulties inherent in present methods of classification pointed out. The gradual growth of a voluminous mass of species, the polymorphism of the organisms as found in culture, the range of spore size, the kaleidoscopic range of colors, all served to make the genus Fusarium a maze of inadequately described species. The appearance of Appel and Wollenweber's monograph (4) established criteria by which the Fusarium species could be properly described and compared. As a result of the indefatigable work of Wollenweber (5), Sherbakoff (6), Reinking and Wollenweber (7), and others, many of the important Fusarium species are now so described that their identification can be made with appropriate study. There still exists with many species extreme difficulty in diagnosis, especially if the culture is not in optimum condition.

As pointed out in the preliminary discussion of the problem, ready diagnosis of species of Fusarium has not been possible for the working pathologist and he, in the main, has needed to rely upon the few who really are specialists in this group of fungi. Recognition of the obstacles to ready determination of species was voiced by the report of a group of specialists who in a conference in 1925 made a forward step in clarification of terms, standardization of media, and closer definition of the sections within the genus (8).

The morphological basis augmented by the description of normal reactions upon standard media is recognized as fundamental for phylogenetic and classification studies. The present paper will attempt to outline auxiliary methods by which, in the species which have been available for study, ready means of diagnosis may be found by using the reactions of the various species of the genus Fusarium to growth-inhibiting agents. The use of such methods in bacteriology has been reviewed in a preliminary publication (2), and lately has been applied to fungi (9, 10).

That some of the relationships in the Fusarium genus which have already been found will reveal themselves anew by similarity of reactions to the growth-inhibiting chemicals may be expected, but that the pattern found on the basis of morphology will be duplicated in a set of physiological reactions is not to be looked for if the specificity shown by organisms is taken into account.

Work on this problem has extended over a considerable period. The

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method used at the beginning was an empirical search for suitable chemicals to which the various species under test would react with a variety of responses. As discussed in the previous reports (1, 2) after test of many substances, the aniline dyes seemed to give the best results and of these the triphenylmethane series seemed most promising.

When suitable dyes were determined, the problem turned to development of a simple methodology whereby adequate constancy of reaction could be obtained. It will be of small account to record the preliminary tests and findings which eventually led to establishment of the technic adopted, but the guiding thought in selecting methods was to choose those which other workers could readily duplicate. The culture method which was developed consists essentially of growing the organisms upon a synthetic agar containing a dye or other chemical. To determine growth limits, a graded series was employed. Cultures were grown in the dark, at definite temperature, with moderate humidity and the reactions recorded by a convenient shorthand method. The following report is based upon the methods finally adopted. The readings given are the ones obtained with the organisms named following these methods with only such deviation as is inherent in ordinary laboratory procedure.

METHODS EMPLOYED

The synthetic medium employed was based upon previous studies by the senior author (3) on a synthetic culture medium. Although the various species of Fusarium apparently are able to oxidize any of the ordinary sugars, dextrose and saccharose were both used in the medium as a sort of insurance that suitable carbohydrate would be present. The early work with various nitrogen compounds had shown that nitrates were readily used by various species of Fusarium as a source of nitrogen, hence potassium nitrate was employed. In a medium intended to foster fruit-body formation it seems desirable to use limited quantities of readily available nutrients to furnish a starvation diet for the organisms after the first flush of growth upon the readily available food stuffs. In contrast to this type of substratum, the synthetic medium used for the present work was devised to give strong vegetative growth and contained many times the food supply of the former. This medium has given a strong growth of mycelium for all species of Fusarium tested and seemed as good as any non-synthetic medium tried. Many species sporulated abundantly on it, but spore production or type of spore mass has not been considered at all in this study, and no record of the sporulation reaction has been taken.

The dyes used for the diagnostic purposes, in order of importance as differentiating agents, are malachite green, crystal violet, brilliant green, and acriflavine. In some later work, interesting reactions to copper sulphate were obtained and the results of preliminary tests with this chemical are included. The bulk of this work has been with the aniline dyes.

The dyes used have been obtained from various sources and were purchased over a considerable range of time. For malachite green, Grübler's (old material purchased in 1911), Grübler's (1922), Coleman and Bell (various samples), and National Aniline Company (various

samples) have all given close approximations in response. The work since 1925 has been done with National Aniline Company material. Crystal violet from Coleman and Bell and from National Aniline Company have been found identical in responses evoked. Brilliant green and acriflavine from only one source have been used (National Aniline Company).

A 0.5 per cent stock dye solution was made in distilled water and kept in a stoppered bottle. Because of the high toxicity, autoclaving or steaming of this solution was not necessary, and no contamination from this source has been found. As will be seen from the schedules given in preparation of the dye agars for pouring of plates, the method used is essentially a graduated dilution system from an original, carefully prepared basal solution of dye in the synthetic agar. By this means, weighing of minute quantities of dye was avoided.

In ordinary procedure when a considerable number of plates were to be poured, the indicated amount of the appropriate stock dye was added to melted synthetic agar and this gave the basal dye concentration. By using different aliquots with melted agar as shown in the schedules, the various dilutions were obtained. This diluting and mixing was done in a 100 or 200 cc. graduate and measuring was fairly accurate. Petri plates were poured, using approximately the same amount of the proper mixture for each plate, and these were allowed to harden upon a level surface.

INOCULUM

The mass of inoculum is a very important factor in securing uniformity of results. In the early work, great variation in response was obtained depending upon whether a large mass of mycelium was used or a scanty amount. Inoculation with spores frequently failed to give growth, whereas a clump of threads of the mycelium used as an inoculum would grow feebly. A chunk from the culture used as inoculum would show a strong growth, usually perched on the bit of agar transferred. To obviate erratic behavior traceable to size or character of the inoculum, the following method was adopted in our work: Coarse, white, cotton thread (Clark's No. 8) was cut in lengths about one and one-half inches long and sterilized in distilled water. These pieces were then laid on a slant of synthetic agar and inoculation made in the usual manner from the culture to be tested. About a week after the growth had overrun the cotton threads, they were lifted from the culture, adhering agar was scraped off, and the threads were cut with sterile scissors into sections about one mm. long. These small sections were used to inoculate a series of dye-agar plates.

Since in our work we were to deal with many cultures, four organisms were planted on the plate, one per quadrant, and the same order of arrangement was followed throughout all the plates of a series. Each test was run in duplicate.

After planting, the plates were placed in stacks in an incubator held at 26° C. (normal variation ½° C.). A shallow pan of water, giving an evaporating surface of about 140 square inches, was placed in the incubator to hold the relative humidity fairly high. Readings were made after 10 days.

PREPARATION OF THE DYE MEDIA

The synthetic agar used throughout the tests had the composition as shown below and was prepared in the usual manner. After the agar was melted by boiling, the solution was filtered through cotton, and sterilized by autoclaving or steaming:

"Vegetative Growth Synthetic Agar"

Saccharose	7.20 grams
Dextrose	3.60 grams
Magnesium sulphate (crystals)	1.23 grams
Potassium acid phosphate	2.72 grams
Potassium nitrate	2.02 grams
Agar (Difco)	12.00 grams
Water	1000.00 grams

The malachite green medium was made by adding the appropriate amount of the stock dye solution to sterile, melted, synthetic agar. The mixture was stirred thoroughly with a sterile glass rod and then the various combinations of the agar plus dye and plain synthetic agar were made as shown in Table 1.

Table 1—Schedules for preparation of the 3 series of malachite green concentrations used in the tests. All dilutions of Stock Dye (0.5 per cent) were made with the 'Vegetative Growth Synthetic Agar'.

Concentration of Malachite Green	Approximate growth-range series		Standard series		Extended series	
	Proportions		Proportions		Proportions	
	Stock dye agar (1×), 10 cc. of 0.5% dye per liter of synthetic agar	Synthetic agar	Stock dye agar (1×), 10 cc. of 0.5% dye per liter of synthetic agar	Synthetic agar	Stock dye agar (2×), 20 cc. of 0.5% dye per liter of synthetic agar	Synthetic agar
	cc.	cc.	cc.	cc.	cc.	cc.
1:2,000,000*	1	99	—	—	—	—
1: 666,666*	3	97	—	—	—	—
1: 400,000*	5	95	5	95	—	—
1: 285,714*	7	93	—	—	—	—
1: 222,222*	9	91	—	—	—	—
1: 200,000	10	90	10	90	—	—
1: 100,000	—	—	20	80	10	90
1: 66,666	30	70	30	70	—	—
1: 50,000	—	—	40	60	20	80
1: 40,000	50	50	50	50	—	—
1: 33,333	—	—	60	40	30	70
1: 28,571	70	30	70	30	—	—
1: 25,000	—	—	80	20	40	60
1: 22,222	90	10	90	10	—	—
1: 20,000	—	—	—	—	50	50
1: 16,666	—	—	—	—	60	40
1: 14,285	—	—	—	—	70	30
1: 12,500	—	—	—	—	80	20
1: 11,111	—	—	—	—	90	10
1: 10,000	—	—	—	—	100	0

*Made more conveniently by diluting the 1:200,000 concentration.

Crystal violet, so far could be judged from percentage solutions, was less toxic than malachite green, hence higher concentrations of this dye were used. Three series of concentrations were employed, called the "Standard," the "Extended" and the "4 times" series. In making the basic stock agar for the standard crystal violet series, 50 cc. of the 0.5 per cent dye was thoroughly mixed with 950 cc. of the "Vegetative Growth Synthetic Agar," and the dilutions made as shown in the body of Table 2. For the Extended series and for the 4-times series, the stock dye agar was made up 2 times and 4 times the strength of the stock dye agar in the Standard Series.

Table 2—Schedules for preparation of the 3 series of crystal violet concentrations used in the tests. All dilutions of the Stock Dye (0.5 per cent) were made with 'Vegetative Growth Synthetic Agar'.

Concentration of Crystal Violet	Standard series		Extended series		"4×" series	
	Proportions		Proportions		Proportions	
	Stock dye agar (1×) made with 50 cc. of 0.5% dye per liter	Synthetic agar	Stock dye agar (2×) made with 100 cc. of 0.5% dye per liter	Synthetic agar	Stock dye agar (4×) made with 200 cc. of 0.5% dye per liter	Synthetic agar
	cc.	cc.	cc.	cc.	cc.	cc.
1:40,000	10	90	—	—	—	—
1:20,000	20	80	10	90	—	—
1:13,333	30	70	—	—	—	—
1:10,000	40	60	20	80	10	90
1: 8,000	50	50	—	—	—	—
1: 6,666	60	40	30	70	—	—
1: 5,714	70	30	—	—	—	—
1: 5,000	80	20	40	60	20	80
1: 4,444	90	10	—	—	—	—
1: 4,000	100	0	50	50	—	—
1: 3,333	—	—	60	40	30	70
1: 2,857	—	—	70	30	—	—
1: 2,500	—	—	80	20	40	60
1: 2,222	—	—	90	10	—	—
1: 2,000	—	—	100	0	50	50
1: 1,666	—	—	—	—	60	40
1: 1,428	—	—	—	—	70	30
1: 1,250	—	—	—	—	80	20
1: 1,111	—	—	—	—	90	10
1: 1,000	—	—	—	—	100	0

In the tests with brilliant green, the various concentrations used were the same as those given in Table 1 for the standard malachite green series.

The various acriflavine concentrations used were the same as those listed in Table 2 for the 4 × crystal violet series.

Copper sulphate was used only in a preliminary way. The concentrations used ranged by a series of 10 even steps from m/100 to m/1000. The strongest concentration m/100 was prepared by dissolving 2.5 grams (1 mole = 249.6 gms.) in a liter of "Vegetative Growth Synthetic Agar" and the other concentrations were prepared from this by diluting with plain synthetic agar.

READING OF RESULTS

Color of mycelium:

From the beginning of this work, the attempt has been made to seek those characters which were outstanding and to utilize them as a means of differentiating the organisms tested. One of the first characteristics which came to attention was the difference in color of mycelium. Production of pigment in Fusarium species in relation to environmental factors has been investigated by Bessey (11). The great majority of the cultures used were white or creamy in their growth on the plain synthetic agar or on the dye agar, but a limited number were immediately recognizable because of definite red color which developed in the mycelium. This characteristic was taken as a readily usable basis of separation and was given first importance in the artificial separation scheme which was employed. Probably other colors could be used for segregating certain species but we found the white and red classes most distinct. It should be noted that the period used, 10 days, is short, and the color character is that associated with the mycelial growth. Any culture which on the plain synthetic medium or on the dye agar was carmine, rose, pink, or rusty-red was considered as belonging to the red group.

Breadth of mycelial mat:

In each test of the Fusaria, there were available for comparison Petri plates of synthetic agar without dye and those with various graduated concentrations of dye. Each series usually employed 10 steps and was in duplicate. It was easy therefore to check the results and to avoid errors which might arise from failure to inoculate, from overheating of inoculum in the course of transfer, or from outside contamination. It may be said that the toxicity of the dye agar is in itself a considerable deterrent to growth of contaminants, and although the work has been carried on in a general laboratory, none but ordinary precautions seem necessary to secure precise results.

Next to color of mycelium, the amount of growth obtained was taken as the most important criterion. As an index of the amount of growth, the diameter of the mat was measured. The reading "0" was used for those cases where no visible extension occurred beyond the original inoculum. Occasionally, a thread or wisp of growth would occur on the inoculum growing above the medium, but no extension into the medium occurred. The growth was recorded as "trace" in all cases where it was less than 0.5 cm. and where it consisted of faint extrusions of the threads into the medium without the production of enough of a mycelial mat to permit close measurement. The term, as used, indicates persistent but discouraged attempts of the organism to grow in the toxic medium.

A mat of mycelium measuring from .5 to 1 cm. in diameter, was recorded as "1". The figure given in each of the subsequent readings for the various organisms represents the reading of the two plates of the given dilution; and, where the two readings did not closely check, the variance is recorded. With the technic adopted, the results of the duplicate plates were almost always in close harmony. Similarly, the other mycelial mat sizes were recorded, as "2", "3", "4", and "5", the figure indicating the breadth to which the mat breadth approached.

Changes in color of medium:

Certain species of Fusarium growing upon the dye agars used have produced a variety of apparently significant changes in the dyes. It is the writers' opinion that this capacity is very useful in diagnosing the species. Attempt has been made to record these various effects, taking into account in each dilution the relative color density of the medium. With the lowest malachite green concentrations, for example, it is not possible to determine clearly whether or not the mycelium has decolorized the dye. In the highest crystal violet concentrations, decoloration may not be complete. Similarly, in these deeper colored plates the intensification of color in the center of the mycelial mat is not always apparent. The findings recorded as ".0" indicate no change and ".1" indicates the production of a halo effect around the culture. In these cases, there is a cleared zone at the periphery of the growth, and a more intense color just outside this zone. The clearest-cut results were shown by the organisms capable of decolorizing the dyes, recorded as ".2". In such cases, the effect was clearly apparent from the under side, the white colony standing out strongly. In some cases, the decolorization of the dye extended in a zone beyond the mycelial growth. Many organisms showed stronger color in the mycelium than in the surrounding medium. For example, organisms growing on crystal violet showed a deeper violet color where the organism was growing. It was thought that the threads had absorbed the dye strongly in these cases. A few other effects upon them were recorded chiefly those in which definite colors appeared in the center of mycelial mat, usually black or blue. These particular manifestations seemed peculiar to certain organisms and were recorded. A few organisms strongly absorbed the dye, and the color was visible in the mycelium as viewed from above. Apparently, the dye taken up in the threads was passed along through the whole of the growth produced.

Edge of the Colony:

In general, the growth produced by the Fusarium tested was circular in outline, and had the typical colony form customary for an organism growing at an even rate out from a center. There were some organisms which characteristically produced a frondose or irregular growth, ".02." The distinction between these two types is probably very definite and usable for the purpose of separation. There is, however, a confusing condition, which arises because of the tendency of certain species to produce definite silky wisps of mycelium which eventually collapse and which also lead to the production of an uneven edge. In the subsequent description of the tests, these cases are indicated as ramose, ".01."

Growth form:

When the work was begun with the toxic dyes, it was thought that the characteristic growth form would be one of the most valuable diagnostic features on which to rely. It was seen later from the comparison of results with various collections of a single species, that this characteristic probably should not be emphasized too strongly. The type of growth is probably the most striking trait of the organism shown in the plate cultures. It seems, however, to be strongly influenced by some undetermined growth tendencies of different strains. In many

cases, it seems somewhat variable, hence this trait was not used except for very close differentiation. For comparative purposes, where a known species or strain can be grown in the same plate with a suspect, the growth form might be useful. For convenience of record, a series of designations to indicate the growth type has been used. Plates 1-7 illustrate these.

The above-mentioned characteristic features found in the cultures of Fusarium were recorded by a shorthand system which proved very convenient in condensing a mass of readings. In this paper, such records are given in the discussion of the results with various cultures to permit the comparison of a series of readings on one accession and of readings obtained from different isolations of what is considered the same species. The number given, however, is not to be in any way considered as a diagnosis of the Fusarium species but should be considered, within its limits, similar to the numerical record formerly used in bacteriology for a convenience in recording of facts with reference to a given bacterial species (12).

NUMBER SCHEME USED FOR RECORDING READINGS OF PETRI-DISH CULTURES

Color of mycelium	
White	10.
Red	20.
Breadth of mycelial mat	
No growth	0
Very slight growth	tr.
.5 cm. to 1 cm.	1.
1 cm. to 2 cm.	2.
2 cm. to 3 cm.	3.
3 cm. to 4 cm.	4.
4 cm. to 5 cm.	5.
Changes in medium color	
No change	.0
Halo	.1
Strong decolorization	.2
Color intensified in mycelium	.3
Edge of colony	
Even	.00
Ramose	.01
Frondose	.02
Growth form of mycelium	
Submerged	.000
Cottony	.001
Villous	.002
Sericeous	.003
Tufted	.004
Submerged, cottony center	.005
Submerged, sericeous center	.006
Submerged, with cottony fringe at edge, atoll	.007
Woolly	.008

CULTURES USED

The cultures used were obtained from various sources. A few trace to early distributions of Fusarium cultures by Department of Agriculture workers. These were accumulated by various students at the Michigan State College and maintained there as stock cultures. The University of Wisconsin, the University of Chicago, and the University of Minnesota kindly sent all their available stock cultures with full data as to the sources. The authors are also indebted to various workers who have furnished cultures. As will be seen, many of the organisms used were the original tubes, or sub-cultures from these, used by the workers at the Fusarium conference (8) and acknowledgement is given to Dr. C. L. Shear and Dr. H. W. Wollenweber for this material. Under the discussion given for each organism, the cultures which are considered representative of the species and variety are listed, and, in some cases, comment is given concerning other organisms received under provisional or apparently incorrect labels. The laboratory number under which the organism was received is listed as an aid to identification of such of these cultures as are now in the hands of various workers. Dr. C. D. Sherbakoff, with a month's intensive work on the cultures used for this work, guided in the selection of representatives of the different organisms by his judgment on the validity of the name assigned to the culture in question. It has been the writers' aim in this already complicated situation to hold fast to the established concepts of the leading specialists as to the particular species and to try to find some simple aids to diagnosis, rather than to confuse an already complicated situation by injection of new or bizarre notions about species limits. As the concept of this or that species is changed by one worker or another, the names will be juggled. The cultures used are the definite basis of this work and the reactions obtained for them are indicated. In so far as the representatives of the species which have been used are adequate and comprehensive, the record given will furnish the typical reactions. In many cases, only a single isolation (frequently the type material) represents the species. The use of the record which is presented, therefore, in such cases, will be limited to a comparison of unknown organisms with this one culture which is the one now considered by specialists as the type of the species or is the culture from which the present current description is drawn.

As the cultures were received the following routine was used. Each culture was fumigated with pyridine as soon as received to avoid introduction of mites which were found annoyingly prevalent in some accessions. Then transfers were made, and, from these sub-cultures, material for dilution plates was obtained. What was apparently a growth from a single spore was isolated from the dilution plates and this was used as the stock culture under a definite accession number. The collection was maintained by transfer made several times during the year and no contamination or mixing of labels occurred during the course of the tests so far as could be detected.

PROVISIONAL KEY TO GROUPINGS

A. Mycelium white

No growth on malachite green 1:400,000 1

Extremely sensitive to malachite green 2
(Growth less than 1 cm. broad and practically restricted to 1:400,000 or 1:200,000 concentration)

Sensitive to malachite green 3
(Growth to about the 1:66,666 concentration, typically not more than 1 cm. broad in the 1:400,000 concentration)

Moderately sensitive to malachite green 4
(Growth usually limited to the lower quarter of the standard range, usually 1–2 cm. broad in the 1:400,000 and 1:200,000 concentrations)

Tolerant of malachite green 5
(Growth in 1:400,000 concentration almost equalling check (3 to 4 cm. broad), or persisting to about midpoint of standard series (1:40,000).)

Highly tolerant of malachite green 6
(Growth persisting through 1:25,000 concentration or beyond)

B. Mycelium red

No growth on malachite green 1:400,000 ..... 1′
Extremely sensitive† to malachite green ..... 2′
Sensitive† to malachite green ..... 3′
Moderately sensitive† to malachite green ..... 4′
Tolerant of malachite† green ..... 5′
Highly tolerant of malachite† green ..... 6′

A. Mycelium white

Page

1. No growth on malachite green, 1:400,000

Growth on malachite green limited to dye concentration 1:2,000,000, or absent

No growth on crystal violet, 1:40,000 . . . *eumartii* 14
Slight growth on crystal violet, 1:40,000 *ensiforme* 14
Slight growth on crystal violet, 1:40,000 *polymorphum* 15
Growth on crystal violet, 1:4,000 ..... *bostrycoides* 15

Growth on malachite green extending to slightly higher concentration (1:666,666)

No growth on crystal violet, 1:40,000 . . . *ossicolum* 15
Slight growth on crystal violet, 1:40,000 (*polymorphum*) .....
Frondose outline ..... *heterosporum* 19
Circular outline ..... *falcatum* 19

†Limits as given for "A."

Growth on crystal violet extended to about 1:20,000 and persisting as "slight" throughout range
1–2 cm. broad in 1:40,000 concentration ..... *cubense* 19
Less than 1 cm. broad in 1:40,000 concentration ..... *subcarneum* 21

Growth on crystal violet (standard series) extending to midrange, about 1:8,000.
Crystal violet not strongly decolorized ..... *camptoceras* 21
Crystal violet decolorized ..... *incarnatum* 23

Growth on crystal violet (standard series) extending beyond midrange
Sericeous, frondose growth ..... *vasinfectum* var. *egyptiacum* 23
Cottony, even-edged growth, crystal violet decolorized ..... *chlamydosporum* 23
Crystal violet only slightly changed. *bullatum* var. *brevius*
bullatum var. *minus* 24

Growth on crystal violet through standard series or slightly beyond, 1:4,000 *neoceras* 24

2. Extremely sensitive to malachite green

(Growth less than 1 cm. broad and practically restricted to 1:400,000 or 1:200,000 concentration)
No growth on crystal violet ..... *sulphureum* 24
Weak growth on crystal violet (Stopping at about 1:6,666) .... *bulbigenum* 25
(Moderately strong growth on crystal violet (Extending to about 1:4,444 concentration))
Strong growth on crystal violet
Sericeous ..... *semitectum* 25
Cottony ..... *filiferum* 25

3. Sensitive to malachite green

(Growth to about the 1:66,666 concentration, typically not more than 1 cm. broad in the 1:400,000 concentration)

Growth on crystal violet restricted, stopping at about 1:6,666 concentration
Crystal violet decolorized in lower concentrations. (Growth-form villous, edge even) ..... *fructigenum* var. *maius* (*niveum*) 28
Crystal violet intensified in the mycelial mat ..... *conglutinans* var. *callistephi* 28

Crystal violet not decolorized
Growth-form typically sericeous or villous ..... *lycopersici* 28
Growth-form typically cottony or submerged ..... *niveum* 33
Growth on crystal violet strong, extending practically through the standard range, (1:4,000); 2 cm. broad in lowest concentration (1:40,000)
Growth-form sericeous ..... *vasinfectum* 33
Growth-form cottony ..... *vasinfectum* var. *inodoratum* 36
Growth on crystal violet extending beyond the standard range (1:4,000)
Growth extending to concentration 1:3,333
Growth-form cottony, frondose. *Gibberella saubinetii*. 36
Growth-form submerged, with sericeous center ..... *conglutinans* 38
Growth extending to concentration 1:2,000
Crystal violet not decolorized. *viride* 38
Crystal violet decolorized .... *striatum* 38
4. Moderately sensitive to malachite green (Growth usually limited to the lower quarter of the standard range, usually 1–2 cm. broad in the 1:400,000 and 1:200,000 concentration)
No growth on crystal violet ..... *acuminatum* 39
Growth on crystal violet limited to first quarter of range (about 1:13,333)
Cottony, no change in medium .... *lini* 39
Submerged, with or without sericeous center
Color usually intensified in mycelium (Brilliant green decolorized) .. *hyperoxysporum* 41
Growth on crystal violet stopping at or near midpoint of standard series (1:6,666)
Growth-form woolly ..... *zonatum* 41
Growth-form sericeous or submerged, with woolly center ..... *sclerotioides* 44
Growth-form sericeous ..... *caudatum* 44
Growth on crystal violet extending beyond midpoint to about 1:5,000 or 1:4,000
Brilliant green not decolorized
Growth-form on crystal violet submerged, with sericeous center ... *orthoceras* 44
Growth-form cottony, even ..... *orthoceras* var. *triseptatum* 45
Brilliant green decolorized
Growth-form on crystal violet cottony ..... *oxysporum* 45

Growth-form villous or sericeous. . *batatatis*†..... 52

Growth on crystal violet extending to 1:2,500 of extended range

Crystal violet not decolorized..... *orthoceras* var. *longius*..... 45

Crystal violet decolorized..... *radicicola*..... 52

5. Tolerant of malachite green

(Growth in 1:400,000 concentration almost equalling check (3 to 4 cm. broad), or persisting to about midpoint of standard series (1:40,000).)

Malachite green not decolorized

Growth on crystal violet limited to standard range; crystal violet not decolorized

Growth-form cottony..... *euoxysporum*..... 53

Growth-form sericeous..... *alluviale*..... 53

Growth-form villous..... (*batatatis*)..... 52

Growth on crystal violet extending to twice normal range, (1:2,000)...

Crystal violet not decolorized..... *moniliforme*..... 56

Crystal violet decolorized..... *malli*..... 56

Malachite green decolorized

Not sensitive to crystal violet

(Growing to approximately 1:2,000, extended series)

Crystal violet decolorized..... *solani*..... 57

solani var. *minus*... 57

6. Not very sensitive to malachite green

(Growth persisting through 1:25,000 concentration or beyond)

Somewhat sensitive to crystal violet

(Growth stopping at about 1:5,000 concentration)..... *martii* var. *viride*.... 57

Tolerant to crystal violet

(Growth extending much beyond standard range)..... *javanicum*..... 58

B. Mycelium on plain synthetic agar, red

1′ No growth on malachite green, 1:400,000. . *tricinctum*..... 58

2′ Extremely sensitive to malachite green

(Growth restricted and limited to 1:400,000 concentration)..... *paspalicola*..... 58

4′ Moderately sensitive to malachite green

(Growth in lower quarter of the standard series 1–2 cm. broad in 1:200,000 concentration)..... *culmorum*..... 62

†c. f. next section.

5′ Tolerant of malachite green
(Growth extending through 1:50,000 concentration)
Growth in lower concentration restricted.....*decemcellulare*..... 62
Growth in lower concentration strong.....*culmorum* var. *leteius* 62

A, Group 1.

Fusarium eumartii Carpenter (Martiella section)

Cultures used:

M. S. C. 200, received February 24, 1924 from R. W. Goss as No. 64.
M. S. C. 215, received April, 1924 from C. D. Sherbakoff as No. 850.

This species as represented by the two cultures named has shown the greatest sensitiveness of any of the Fusaria to aniline dyes. In repeated tests, no growth was obtained in concentrations as low as 1 to 2,000,000 of malachite green or in crystal violet 1 to 40,000. When brilliant green was used, growth was restricted and limited to the lowest concentration used, 1:400,000. Growth was likewise inhibited by acriflavine, only slight indications of growth occurring in the lower dilutions, 1:10,000 and 1:3,333 (Table 3).

When copper sulphate was used as the toxic substance in the synthetic agar, the limit of growth was at m/800, a concentration corresponding to 1:3,235. In this concentration, Accession 215 produced a white, submerged growth, circular in outline between 1 and 2 centimeters broad (12.000). In slightly higher dilution m/900, the growth was broader but otherwise the same, (13.000). Accession 200 produced only a trace of growth in these concentrations of copper sulphate.

Accession 205, received under the label *eumartii* from R. W. Goss as No. 48 on February 4, 1924, which was originally obtained from Thurston as Pennsylvania State College 62, has not been found to conform to the behavior given for the two preceding cultures which we have taken as typical of the species. This organism has greater tolerance of malachite green, growing in concentrations up to 1:200,000 producing strong decolorization. It has similarly shown great tolerance of crystal violet, growing almost throughout the standard series used for this dye. It has shown considerable tolerance of brilliant green, acriflavine, and copper sulphate. Morphological study of the culture by C. D. Sherbakoff indicated that the organism belonged to the Martiella group. The reactions we have obtained have most nearly approached those obtained from Accession 313, which was provisionally assigned to *Fusarium solani*. See Plate I.

Fusarium ensiforme Wr. and Reinking (Martiella section)

Culture used:

M. S. C. 296, isolation from rotted fig, received from C. L. Shear as No. 876; used by Fusarium conference as type.

This organism as represented by the single accession available closely parallels *eumartii* in its sensitiveness to dyes but may be slightly more tolerant of crystal violet. The record obtained in our trials is shown in Table 4.

Our experience therefore indicates sensitiveness in all dilutions up to 1:2,000,000 of malachite green. In this extremely high dilution, growth was white, less than 1 cm. broad, no effect on the medium could be read because of paleness of the culture medium due to extreme dye dilution, the edge was even, and the growth was cottony. On crystal violet standard series, the growth on none of the concentrations was broad enough to measure, but consisted merely of small wisps of mycelium pressing out from the inoculum, but this feeble growth persisted throughout the series. Other dyes were not tried. See Plate I.

Fusarium polymorphum Matr. (Discolor section)

Culture used:

M. S. C. 265, obtained from H. W. Wollenweber as No. 1250.

This organism was extremely sensitive to malachite green growing only in the lowest concentration used (1:2,000,000) and then very weakly. The growth was white, cottony, circular, and less than 1 cm. broad. It was similarly sensitive to crystal violet in the standard series, no growth resulting except slight wisps of mycelium too small to measure which developed in the second step of the series (1:20,000) in one test. In other tests, no growth was obtained, (Table 4). On the plain synthetic agar, the growth was recorded as 13.001.

Fusarium bostrycoides Wr. and Reinking (Elegans section)

Culture used:

M. S. C. 291, from soil, Honduras, received from C. L. Shear, as No. 866.

The single accession representing this species was very sensitive to malachite green, growth in the extreme concentrations 1:2,000,000 and 1:400,000, being limited to a trace. On crystal violet, growth was strong throughout all concentrations of the standard series, typically producing strong decolorization of the medium. The growth-form was ramose and the growth was typically sericeous or submerged with sericeous center. Representative readings obtained with this culture are given in Table 4.

This organism, which is encountered in the same habitat as *Fusarium cubense,* probably could be distinguished from *F. cubense* by its weaker growth in the plain synthetic agar medium and by its ability to grow on and to decolorize crystal violet in the standard series.

Fusarium ossicolum (Berk. & Curtis) Sacc. (Gibbosum section)

Culture used:

M. S. C. 307, isolated from banana leaves, Jamaica, received from C. L. Shear as No. 896.

This culture was very sensitive to malachite green, growing only on the lower concentrations used, 1:2,000,000 and 1:666,666. The growth was white in each case, 2 or 3 cm. broad, depending on the degree of concentration; no decolorization was detectable; the edge of the colony was frondose; and the growth form was cottony. The organism seemed

Table 3—Reactions of *Fusarium eumartii* (Accessions 200 and 215) to various concentrations of aniline dyes and copper sulphate in synthetic agar, and to synthetic agar without toxic substances.

Malachite Green Concentrations

Accession Number	Date of Test	1:2,000,000	1:666,666	1:400,000	1:285,714	1:222,222	1:200,000	1:66,666	1:40,000	1:28,571	1:22,222
200	Mar. 10, 1927	—	—	0	—	—	0	—	0	—	0
200	Apr. 21, 1927	—	—	tr	—	—	0	0	0	0	0
200	Apr. 29, 1928	—	—	—	—	—	0	—	0	—	0
200	May 1, 1929	0	0	0	0	0	0	0	0	0	0
215	Mar. 10, 1927	—	—	0	—	—	0	0	0	0	0
215	Apr. 21, 1927	—	—	tr	—	—	tr	tr	0	0	0
215	Apr. 29, 1928	—	—	—	—	—	0	—	0	—	0
215	May 1, 1929	0	0	0	0	0	0	0	0	0	0

Crystal Violet Concentrations

Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
200	May 13, 1927	0	0	0	0	0	0	0	0	0	0
200	Apr. 29, 1928	—	0	—	0	—	0	—	0	—	0
200	May 4, 1929	0	—	0	—	0	—	0	—	0	—
215	May 13, 1927	0	0	0	0	0	0	0	0	0	0
215	Apr. 29, 1928	—	0	—	0	—	0	—	0	—	0
215	May 4, 1929	0	—	0	—	0	—	0	—	0	—

Brilliant Green Concentrations

Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
200	July 3, 1927	11 006	0	0	0	0	0	0	0	0	0
215	July 3, 1927	11 006	tr	0	0	0	0	0	0	0	0

Table 3—Continued.

Acriflavine Concentrations

Accession Number	Date of Test	1:10,000	1:5,000	1:3,333	1:2,500	1:2,000	1:1,666	1:1,428	1:1,250	1:1,111	1:1,000
200	Apr. 29, 1928	tr	—	tr	—	0	—	0	—	0	—
215	Apr. 29, 1928	tr	—	tr	—	0	—	0	—	0	—

Copper Sulphate Concentrations

Accession Number	Date of Test	m/1000	m/900	m/800	m/700	m/600	m/500	m/400	m/300	m/200	m/100
200	Jan. 27, 1929	tr	tr	tr	0	0	0	0	0	0	0
215	Jan. 27, 1929	13.000	13.000	12.000	0	0	0	0	0	0	0

Synthetic Agar without Toxic Substance

200	Apr. 29, 1928	13.005
215	Apr. 29, 1928	13.005
200	May 1, 1929	12.008
215	May 1, 1929	13.007

Table 4—Comparative readings obtained from several species of Fusarium of 'A, Group 1' growing on synthetic medium with a graded series of malachite green and crystal violet concentrations.

Malachite Green Concentrations

Species	Accession Number	Date of Test	1:2,000,000	1:666,666	1:400,000	1:285,714	1:222,222	1:200,000	1:66,666	1:40,000	1:28,571	1:22,222
F. ensiforme	296	Feb. 10, 1927	—	—	0	—	—	0	0	0	0	0
F. ensiforme	296	May 1, 1929	11.—01	0	0	0	0	0	0	0	0	0
F. polymorphum	265	Apr. 20, 1929	11.—01	tr	0	0	0	0	0	0	0	0
F. bostrycoides	291	Mar. 10, 1927	—	—	tr	—	—	tr	0	0	0	0
F. bostrycoides	291	Apr. 20, 1929	tr	0	0	0	0	0	0	0	0	0
F. ossicolum	307	Mar. 10, 1927	—	—	tr	—	—	0	0	0	0	0
F. ossicolum	307	Apr. 20, 1929	13.021	12.021	0	0	0	0	0	0	0	0
F. heterosporum	272	Apr. 28, 1929	—	—	—	—	—	0	0	0	0	0
F. falcatum	297	Mar. 10, 1927	—	—	0	—	—	0	0	0	0	0
F. falcatum	297	Apr. 28, 1929	—	—	—	—	—	0	0	0	0	0

Crystal Violet Concentrations

Species	Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
F. ensiforme*	296	Feb. 10, 1927	tr	tr	0	tr	0	tr	tr	tr	tr	tr
F. polymorphum	265	June 28, 1925	0	tr	0	0	0	0	0	0	0	0
F. bostrycoides	291	June 21, 1925	12.213	12.213	12.206	11.206	11.206	11.213	11.213	11.213	11.213	11.213
F. bostrycoides	291	Feb. 8, 1927	12.213	12.213	11.213	11.213	11.213	11.213	11.213	11.213	11.213	11.213
F. ossicolum	307	Feb. 8, 1927	11.022	—	0	tr	0	11.022	tr	tr	tr	tr
F. ossicolum	307	May 3, 1929	—	0	—	0	—	0	—	0	—	0
F. heterosporum	272	July 24, 1925	0	0	0	0	0	0	0	0	0	0
F. heterosporum	272	Feb. 8, 1927	0	11.012	0	0	0	0	0	0	0	0
F. falcatum	297	Feb. 10, 1927	11.001	11.002	0	0	tr	0	0	tr	0	0
F. falcatum	297	May 3, 1929	—	0	—	0	—	0	—	0	—	0

*Readings made April and June, 1925 correspond with these.

sensitive to crystal violet as determined by the test of May 3, 1929. On certain previous tests, there had been a weak growth on the lowest dilutions of the standard series. On plain synthetic agar, the growth was white, strong (5 cm. or more broad) with even edge, and of cottony type. The record is given in Table 4. See Plate V.

Fusarium heterosporum Nees. (Discolor section)

Culture used:

M. S. C. 272, obtained from H. W. Wollenweber as No. 1479.

With this organism, usually no growth occurred in any of the steps of the standard malachite green series. In a few instances, slight development of wisps of mycelium too small to measure occurred in the lower steps. On the crystal violet standard series, growth developed in one test in the second step containing crystal violet, 1:20,000. The growth was white, about 1 cm. broad and no discolorization of medium noticeable. The mat was typically ramose in outline and villous in growth-type (12.012). Occasionally, other readings showed growth-type as cottony (Table 4).

Fusarium falcatum Appel and Wr. (Gibbosum section)

Culture used:

M. S. C. 297, obtained from C. L. Shear as No. 879, originally isolated from soil in Honduras.

With this organism, no growth was obtained on any concentration of the standard series containing malachite green. When crystal violet was used, in one series, growth stopped at the second step of standard series (1:20,000). The growth was white, about 1 cm. broad, no decolorization was produced, the edge was even, and the growth-type cottony or villous (11.001 or 11.002). In another test growth was inhibited at 1:20,000 dilution. On plain synthetic medium, the growth was white, 4-5 cm. broad, circular in outline, and either submerged or submerged with coarse woolly outgrowths at the center (14.000 or 14.008). It is probable that the reactions on synthetic media and the dye agars would only be needed in the case of this organism to supplement decision based on spore form and sizes.

Fusarium cubense E. F. Smith (Elegans section)

Cultures used:

M. S. C. 425, isolated by O. A. Reinking in Honduras and called by him R 3 (obtained from C. D. Sherbakoff);
M. S. C. 429, isolated by Reinking in Honduras and called by him R 15 (from C. D. Sherbakoff);
M. S. C. 436, obtained from C. D. Sherbakoff through M. A. Carleton (originally from Honduras).

All of these accessions were very sensitive to malachite green, producing no growth in standard series except the faint suggestion of wisp-like growth pushed out around the inoculum. On crystal violet, the growth was white, stopping sharply at the second or third step of the standard series (1:20,000), except for a mere trace of growth

Table 5—Readings of *Fusarium cubense* (three accessions) on synthetic agar containing graded concentrations of malachite green and of crystal violet. Period 10 days.

Malachite Green Concentrations

Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
425	Mar. 17, 1928	tr	0	0	0	0	0	0	0	0	0
425	Apr. 27, 1929	—	0	—	0	—	0	—	0	—	0
429	Mar. 17, 1928	tr	0	0	0	0	0	0	0	0	0
429	Apr. 27, 1929	—	tr	—	0	—	0	—	0	—	0
436	Mar. 17, 1928	tr	0	0	0	0	0	0	0	0	0
436	Apr. 27, 1929	—	0	—	0	—	0	—	0	—	0

Crystal Violet Concentrations

Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
425	Mar. 17, 1928	11.005 11.001	11.001	tr	tr	tr	tr	tr	tr	tr	tr
425	May 3, 1929	—	tr	—	tr	—	tr	—	0	—	0
429	Mar. 17, 1928	12.013	12.013	tr	tr	tr	tr	tr	tr	tr	tr
429	May 3, 1929	—	11.001	—	11.003	—	tr	—	0	—	0
436	Mar. 17, 1928	12.013	12.013 12.000	tr	tr	tr	tr	tr	tr	tr	tr
436	May 3, 1929	—	11.003	—	11.—	—	tr	—	0	—	0

Synthetic Agar without Dye

425		14 001
425		14 001
425		14 001
429		14 001
429		14 001
436		14 007
436		14 000
436		14 001

which persisted in the higher concentration. On plain agar, the growth was strong (4-5 cm. broad), of even edge, and cottony growth habit. See Plate I. Typical records for these organisms are given in Table 5.

The following other organisms received under the label *Fusarium cubense* were studied: M. S. C. 227, received April 20, 1924 from C. D. Sherbakoff as No. 887-h from Serrano, P. I. This organism grew strongly in the first two steps of the standard malachite green series giving readings 13.001 and 11.001, respectively. On malachite green (approximate-growth-range series), growth persisted through the sixth step of the series (1:200,000) (corresponding to step 2 of the standard series), giving the following readings: 14. -13; 12. -13; 11. -13; 11. -13; 11. -13; 11. -13; tr; 0; 0; 0 for the 10 respective steps. This accession had more tolerance for malachite green than the other three believed to be typical *F. cubense.* On plain synthetic agar, the readings were 14.007 and 14.000. It was not tested on crystal violet. Its identity with *F. cubense* was not accepted by Sherbakoff on morphological grounds.

M. S. C. 424, received under the name *F. cubense,* isolated by Dean from Honduras material (from Aboca) was received from C. D. Sherbakoff under No. 1069.9 This organism gave typical reaction on malachite green with those accepted as types, but in crystal violet growth persisted through the eighth step, and the organism decolorized crystal violet. It was believed that this organism is closer to *F. bostrycoides* in its reactions. Strain 285, isolated from vascular bundles of banana in Honduras and received from Shear as No. 867, gave strong growth in the first step of the standard malachite green (13.001) and weaker growth in the next two steps (11.001). On crystal violet, growth though weak persisted through the fifth step of the standard. This organism had greater tolerance to malachite green than those accepted as types and its trueness to label is questioned. Unfortunately, this culture was lost and its identification could not be followed.

Fusarium subcarneum Crouan (Discolor section)

Culture used:

M. S. C. 260, obtained from H. W. Wollenweber, July, 1924, as No. 1427.

This organism produced no growth in the standard malachite green series and in the series using lower concentrations the growth was limited to the two lowest concentrations corresponding to 1 :2,000,000 and 1 :666,666, respectively. The growth on these two extreme dilutions was white, about 1 cm. broad; no decolorization was detectable, the edge was even, and the growth-form villous or sericeous. On crystal violet, the growth was restricted, stopping at the third or fourth step, but slight evidence of growth (less than 1 mm.) was shown in the higher dilutions. The record for this organism is given in Table 6.

Fusarium camptoceras Wr. and Reinking (Arthrosporiella section)

Culture used:

M. S. C. 295, obtained from C. L. Shear as No. 873, originally isolated from rotting cacao pod, Honduras.

Table 6—Comparative readings obtained from several species of Fusarium ('A, Group 1') growing on a graded concentration series of malachite green and crystal violet in synthetic agar. Period 10 days.

Malachite Green Concentrations

Species	Accession Number	Date of Test	1:2,000,000	1:666,666	1:400,000	1:285,714	1:222,222	1:200,000	1:66,666	1:40,000	1:28,571	1:22,222
F. subcarneum	260	Apr. 20, 1929	11.002	11.003	tr	0	0	0	0	0	0	0
F. camptoceras	295	Apr. 14, 1929	—	—	—	—	—	—	0*	0	0	0
F. incarnatum	281	Apr. 20, 1929	11.001	11.001	0	0	0	0	0	0	0	0
F. vasinfectum var. egyptiacum	435	Mar. 17, 1929	—	—	tr	—	—	tr	0	0	0	0
F. chlamydosporum	276	Apr. 20, 1929	tr	tr	tr	0	0	0	0	0	0	0
F. chlamydosporum	276	May 1, 1929	11.-01	tr	0	0	0	0	0	0	0	0
F. bullatum var. brevius	286	Apr. 6, 1929	—	—	—	—	—	0	0	0	0	0
F. bullatum var. minus	289	Apr. 6, 1929	—	—	—	—	—	0	0	0	0	0
F. neoceras	308	Apr. 20, 1929	12.001	11.001	0	0	0	0	0	0	0	0

Crystal Violet Concentrations

Species	Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
F. subcarneum	260	Feb. 8, 1927	11.013	11.013 12.012	tr	0	tr	tr	tr	—	0	tr
F. subcarneum	260	May 3, 1929	—	11.012	—	11.- -	—	tr	—	tr	—	0
F. camptoceras	295	Feb. 8, 1927	11.012	11.012	11.112	11.112	tr	tr	tr	tr	tr	tr
F. camptoceras	295	May 3, 1929	—	tr	—	tr	—	tr 0	—	0	—	0
F. incarnatum	281	Jan. —, 1925	12 111	12 111	12 311	11.311	11 —1	11 —1	tr	tr	tr	tr
F. incarnatum	281	Apr. 2, 1925	12.213	12 213	11 211	11 211	11 211	11 211	11 213	tr	tr	tr
F. incarnatum	281	June 20, 1925	11 201	11.211	11 211	11.211	11 211	11 211	0	tr	tr	tr
F. vasinfectum var. egyptiacum	435	Mar. 17, 1929	12.013	11 013	11 013	11 013	11 013	11 013	11 013	11 013	11 013	11.013
F. vasinfectum var. egyptiacum	435	May 3, 1929	—	11.013	—	11.013	—	11.013	—	tr	—	0
F. chlamydosporum	276	June 20, 1925	11 201	11 201	11 201	11.201	11 201	11.201	11.201	11 201	tr	0
F. bullatum var. brevius	286	Jan. —, 1925	12.501	12.501	11 -01	11.501	11 -01	11 -01	tr	tr	tr	tr
F. bullatum var. minus	289	May 3, 1929	—	11.001	—	11.001	—	tr	—	tr	—	0
F. neoceras	308	Mar. 19, 1929	—	11.002	—	11.002	—	11.002	—	11.002	—	11.002†

*No growth, 1:100,000.
†Growth continued through 1:3,333 concentration.

No growth was obtained on standard series malachite green, except slight growth in a few cases at the 1:400,000 concentration. On crystal violet, standard series, the growth was very weak, measurable growth stopping at about the fourth step (1:10,000), but with a trace (less than 1 mm.) growth persisting throughout the series. The growth in the first four concentrations was white, about 1 cm. broad, and showed slight tendency to decolorize. The edge was ramose and the growth-form was villous. (11.112 is fairly typical reading for steps 3 or 4.) On the plain synthetic agar, the reading was 14.001. The growth-form was appressed cottony.

Fusarium incarnatum (Rob.) Sacc. (Arthrosporiella section)

Culture used:

M. S. C. 281, received from C. L. Shear as No. 878.

This organism did not grow on any concentration of the standard malachite green series. On malachite green, 1:2,000,000 and 666,666, a weak white growth was produced, less than 1 cm. broad, with even edge, cottony type. Decolorization could not be determined. (11. -01.) On crystal violet, the growth was somewhat restricted even in the lowest concentrations and was stopped at a step or two beyond mid-point in the standard series. The growth was white, about 1 cm. broad, crystal violet was decolorized, the edge was uneven, and the growth-form cottony. On the plain synthetic agar, the growth was moderate (2-3 cm. broad), even, cottony (Table 6).

Fusarium vasinfectum Atk. var. *egyptiacum* Fahmy (Elegans section)

Culture used:

M. S. C. 435, received from C. L. Shear as No. 953; originally from Egypt (Fahmy), No. 819.

This organism, as represented by the single culture available, showed slight growth on the standard malachite green series ("tr"). The growth on crystal violet was moderate and, in one series, persisted throughout the range. The growth was white, 2 cm. broad in the lowest dilution used, then 1 cm. broad (average) in all others, and persisted practically through the range. The medium was not decolorized, the edge was ramose, and the growth-form sericeous. A typical reading throughout crystal violet range was 11.013. On the plain synthetic agar, the reading was 14.000.

Fusarium chlamydosporum Wr. and Reinking (Sporotrichella section)

Culture used:

M. S. C. 276, received from C. L. Shear as No. 871, isolated from banana, Honduras.

This organism was extremely sensitive to malachite green, producing only weak growth in the lowest dilutions used (1:2,000,000). It is restricted in its extension by crystal violet, growth stopping at the sixth to eighth step of the standard series. On crystal violet, the growth was white, about 1 cm. broad; the dye was strongly decolorized. The edge of the growth was even and the growth-form cottony. On plain

synthetic agar, the growth was white, 3 cm. or more broad, with uneven edge, and cottony growth-form (Table 6).

Fusarium bullatum Sherb. var. *brevius* Wr. and Reinking (Gibbosum section)

Fusarium bullatum Sherb. var. *minus* Wr. and Reinking

Cultures used:

M. S. C. 286, (*brevius*) isolated from trash in Honduras, received From C. L. Shear as No. 864;

M. S. C. 289, (*minus*) isolated from plant trash in Jamaica, received from C. L. Shear as No. 8630.

No growth from either variety on malachite green of the standard series except weak or slight growth in the first step (1:400,000) in a few early tests where quantity of inoculum may have been a factor. On crystal violet, the variety *brevius* produced slight growth at the seventh step (1:5,714). The variety *minus* grew enough to be measured (.5 to 1 cm. broad) in the fourth step or fifth step (1:10,000) with slight growth in the higher concentrations. It is not sure that these small differences in reaction to crystal violet are sufficient to permit differentiation of the varieties. No culture of the species originally described by Sherbakoff was available.

Fusarium neoceras Wr. and Reinking (Liseola section)

Culture used:

M. S. C. 308, received from C. L. Shear as No. 8970, isolated from soil in Honduras.

This organism produced slight or, at most, weak growth in the lowest concentration of the standard series. In the malachite green series, using lower concentrations, (approximate-growth-range series) growth occurred in the lowest concentrations (1:2,000,000 and 1:666,666) but none was obtained in concentration of 1:400,000. The organism was very tolerant of crystal violet, growing in all concentrations of the standard series; with tests using higher concentrations, growth was obtained in a concentration of 1:3,333. The growth was white; about .5 to 1 cm. broad, depending on the concentration; produced no decolorization but, in some cases, the color was intensified in the mycelium; the edge was even, and the growth-form appressed cottony or villous.

The reactions of this organism are probably sharp enough to characterize it clearly, but the natural purple color of the stroma produced on various media, including the synthetic agar used in this study, would undoubtedly serve readily to identify this organism from others. See Plate V.

A, Group 2.

Fusarium sulphureum Schlecht. (Discolor section)

Culture used:

M. S. C. 250, received in August, 1924, from H. W. Wollenweber as No. 1642.

On malachite green, standard series, this organism produced on the lowest concentration (1:400,000) a white growth, 1 cm. broad. No decolorization was discernible, the edge was even, and the growth-form cottony (11.001). On crystal violet, the organism gave no growth in the second step (Table 7). On plain synthetic agar the growth was white, strong 4-5 cm. broad), with even edge, and cottony (14. -01).

Fusarium bulbigenum Cooke and Massee (Elegans section)

Cultures used:

M. S. C. 284, received from C. L. Shear as No. 867, isolated from banana in Honduras;

M. S. C. 172, received from G. K. K. Link, February 4, 1924, as No. 1634; originally from Wollenweber as No. 1452.

These two organisms, apparently morphologically and culturally are near enough alike to warrant their inclusion under the same name (*fide* Sherbakoff) have not given identical response with the toxic media, but the reactions need further checking. The detailed results obtained are recorded without comment, pending additional readings. It is believed that variance in reading is more attributable to the technic than to fundamental differences in the two cultures (Table 7). On plain synthetic agar, the growth of both accessions was white, 4 cm. broad, circular in outline, and typically submerged or submerged with cottony center.

Fusarium semitectum Berkeley and Ravenel (Arthrosporiella section)

Cultures used:

M. S. C. 306, received from C. L. Shear as No. 895;

M. S. C. 333, received from International Bureau for Fungus Cultures (Holland) through C. L. Shear as No. 945.

These two organisms have shown fairly similar sensitiveness to malachite green, the lowest concentration permitting growth being somewhere between the first and second steps of the standard series (between 1:400,000 and 1:200,000). There is considerable difference in the growth reactions of the two organisms as will be seen by the readings given. On crystal violet, the differences are great enough to bring questions as to the identity of the two organisms bearing this label which Sherbakoff found to conform to the morphological and other cultural criteria for the species. The record is given to put on record the situation found (Table 7). On plain synthetic agar, the reading for M. S. C. 306 was 12.000 and for M. S. C. 333, 13.021. The latter produced abundant masses of pink spores on the plain medium.

Fusarium filiferum (Preuss.) Wr. (Gibbosum section)

Culture used:

M. S. C. 252, received from Wollenweber in July, 1924, as No. 3750.

This organism has grown only on the lowest steps of the standard series (1:200,000), producing a weak growth, white, .5 to 1 cm. broad, no decolorization discernible, edge even, growth-form cottony (11.001).

Table 7—Reactions of Fusaria of 'A, Group 2' to various concentrations of malachite green and crystal violet in synthetic agar.

Malachite Green Concentrations

Species	Accession Number	Date of Test	1:2,000,000	1:666,666	1:400,000	1:285,714	1:222,222	1:200,000	1:66,666	1:40,000	1:28,571	1:22,222
F. sulphureum	250	Mar. 10, 1927	—	—	11.001	—	—	0	0	0	0	0
F. sulphureum	250	Apr. 28, 1929	—	—	—	—	—	0	0	0	0	0
F. bulbigenum	284	Apr. 20, 1929	13 016 13 013	12 013	12 013	11 003	11.000	11.000	0	0	0	0
F. bulbigenum	172	Apr. 20, 1929	11.013	11 —	tr	0	0	0	0	0	0	0
F. semitectum	306	Apr. 20, 1929	11 001	tr	tr	11.001	0	0	0	0	0	0
F. semitectum	333	Apr. 20, 1929	12 023	11 023	11 023	11 023	11.023	0	0	0	0	0
F. filiferum	252	Apr. 27, 1929	—	—	—	—	—	11.001 tr	0	0	0	0
F. filiferum	252	Apr. 14, 1929	—	—	—	—	—	tr	0	0	0	0

Crystal Violet Concentrations

Species	Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
F. sulphureum	250	May 3, 1929	—	0	—	0	—	0	—	0	—	0
F. bulbigenum	284	May 3, 1929	—	11. 01	—	11.-01	—	11.-01	—	tr	—	tr
F. bulbigenum	172	May 3, 1929	—	tr	—	11.213	—	0	—	0	—	0
F. semitectum	306	May 3, 1929	—	0	—	0	—	0	—	0	—	0
F. semitectum	333	May 3, 1929	—	12.223	—	12.223	—	12.223	—	11.223	—	11.023
F. filiferum	252	May 3, 1929	—	12.211	—	12.211	—	11.211	—	11.211	—	tr 0

Table 8—Reactions of various Fusaria of 'A, Group 3', to various concentrations of malachite green and of crystal violet in synthetic agar.

Malachite Green Concentrations

Species	Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
F. fructigenum var. maius	288	Apr. 27, 1930	—	11.011	—	11.011 0	—	0	—	0	—	0
F. conglutinans var. callistephi	58	Apr. 27, 1930	—	11.013	—	0	—	0	—	0	—	0
F. niveum	430	Mar. 15, 1928	11.000	11.000	tr	0	0	0	0	0	0	0
F. niveum	430	Apr. 27, 1929	—	11.006	—	11.001	—	tr	—	0	—	0
F. niveum	418	Mar. 15, 1928	11.013 11.001	11.013	0	0	0	0	0	0	0	0
F. niveum	418	Apr. 27, 1929	—	11.001	—	0 11.001	—	0	—	0	—	0
F. niveum	421	Mar. 15, 1928	tr	0	0	0	0	0	0	0	0	0
F. niveum	421	Apr 27, 1929	—	0	—	0	—	0	—	0	—	0

Crystal Violet Concentrations

Species	Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
F. fructigenum var. maius	288	June 20, 1925	11.202	12.205	11.–02	11.–02	11.–02	tr	tr	tr 0	0	0
F. conglutinans var. callistephi	58	June 23, 1925	11 311	11.311	11 311	11 311	11 311	11 311	tr	tr	0	0
F. niveum	430	Apr. 16, 1928	11 000	11 000	11 000	11 000	11 000	11 000	11.000	11 000	11 000	tr
F. niveum	430	May 3, 1929	—	11 303	—	11 301	—	11 .01	—	11 –01	—	tr
F. niveum	418	Apr. 16, 1928	13 202	13 202	13.213	13.201	13.213	13.213	13.213	13.213	13.213	12.201
F. niveum	418	May 3, 1929	—	13.201	—	12.201	—	12.201	—	12.201	—	11.101
F. niveum	421	Apr. 16, 1928	12 001	12 001	11 001	11 001	11 001	11.001	11 001	11.001	11.001	0 11.–01
F. niveum	421	May 3, 1929	—	11.301	—	11.301	—	tr	—	0	—	0

On the crystal violet, standard series, growth extended almost through the range of the series but was restricted somewhat by the dye. The organism decolorized crystal violet. The growth is white, 2 cm. or more broad at the midrange, then 1 cm. or less; the edge is ramose, and the growth-form cottony. On plain synthetic agar, the growth was white, 4 cm. broad, with edge even, and growth-form cottony (14.-01).

A, Group 3.

Fusarium fructigenum Fr. var. *maius* Wr. (Lateritium section)

Culture used:

M. S. C. 288, received December, 1924, from C. L. Shear as No. 877; isolated from plant debris, Jamaica.

This organism has produced restricted growth on the second and the fourth steps of the standard malachite green series (1:66,666), being less than 1 cm. broad, white, with no decolorization produced. The edge typically was uneven and the growth-form cottony (11.011). On crystal violet, growth has stopped at a little beyond midrange of the standard series. The growth is restricted throughout the range, being between .05 and 1 cm. broad. The organism decolorized crystal violet slightly, this being evident only in the lower dilutions (11.202). The edge is even, and the growth-form typically villous or submerged with cottony or coarse cottony center (Table 8). On plain agar, the reading was 14.008.

Fusarium conglutinans Wr. var. *callistephi* Beach (Elegans section)

Culture used:

M. S. C. 58, original type culture of W. S. Beach.

On malachite green, standard series, restricted growth in the first two steps (extending to the third or fourth, with heavy inoculation). Growth in second step (1:200,000), white, 1 cm. broad, no decolorization discernible, edge ramose, growth-form sericeous (11-13). On crystal violet, standard series, growth was restricted even in the lowest concentrations. The mycelial mat was 1-2 cm. wide in the lowest concentrations, and from 0.5 to 1 cm. in the higher concentrations. Growth is checked about the sixth step persisting as a trace nearly through the range. The crystal violet is changed from purple to blue by the organism, and the dye seems absorbed in the mycelial mat, the edge of the growth is irregular, and the growth-form cottony (11.311) (Table 8). On plain synthetic media, the reading was 14.000 or 14.008.

Fusarium lycopersici (Sacc.) Wr.. (Elegans section)

Cultures used:

M. S. C. 170, received from G. K. K. Link, February 4, 1924, as No. 1518; obtained from Shapavalov as F. J. Pritchard's No. 103;

M. S. C. 393, received from R. P. White, June 27, 1927, as Kansas No. 60;

M. S. C. 259, received from Wollenweber, July 19, 1924, as No. 5001;

M. S. C. 408, received from R. P. White as Kansas No. 68;
M. S. C. 153, received from C. D. Sherbakoff, January 28, 1921, as No. 17;
M. S. C. 210, received from L. R. Jones, March 20, 1924, as No. 317; originally from Clayton.
M. S. C. 411, received from R. P. White, June 27, 1927, New Jersey isolation, as Kansas No. 128;
M. S. C. 434, received from C. D. Sherbakoff as No. 998, isolated from wilted tomato plants from South Carolina;
M. S. C. 437, received from C. D. Sherbakoff, isolated from wilted tomato, (western Tennessee);
M. S. C. 438, received from C. D. Sherbakoff as No. 8995, originally from Shapavalov's California isolation and reported as very pathogenic.

The results of a series of cultures using these strains with various dyes can best be shown in a series of tables, and following this a generalized statement of limits of the species as shown by these cultures is given (Table 9).

A generalized statement covering the reactions obtained is given: On malachite green, standard series, the organism shows sensitiveness to the dye, growth being restricted to the two lowest steps, 1:200,000 and 1:400,000. In the case of some isolations only slight growth is shown in these concentrations. The mycelium color is white, typically 1 cm. across or less, no decolorization is discernible on account of the high dilution of the plates, the edge is even and the growth-form villous, cottony, or sericeous.

On crystal violet, an average of all the strains tested would place the limit of tolerance of the series of dilutions used as a standard, at between the sixth and ninth step usually before the eighth step (1:5,000). It is probable that variations in technic are in part responsible for the variations in response. The growth on crystal violet is restricted and is, even in the lowest concentrations, not greater than 2 cm. broad, being prevailingly about 1 cm. One or two organisms intensified the color in the mycelium, notably Accession 210. The edge of the growth is probably typically even but in a few cases, especially where the growth-form is sericeous, the falling of these sericeous wisps of mycelium has made the outline of the colony somewhat uneven. No prevailing growth-form was found but in general the aerial mycelium was coarse and tended to clump.

The reactions with brilliant green used in the same percentage concentrations as malachite green, were sharp, and may be useful in differentiating this organism.

Accession 210, when used on synthetic agar containing copper sulphate, in a graded series of 10 dilutions ranging from m/1000 to m/100, showed slight growth, too small for measurement in m/200 and m/300 concentrations, and strong growth in m/400 (11.002). The growth became strong as the dilution increased and in m/700 the breadth of mycelium was 3 cm. *F. lycopersici*, as represented by the culture, seems fairly tolerant of copper sulphate, and this characteristic may be useful as an additional aid in diagnosis. On plain synthetic agar all the organisms grew strongly, with even edge, and showed in general woolly or villous mycelium. See Plate III.

Table 9—Reactions of various accessions of *Fusarium lycopersici* to various concentrations of malachite green, of crystal violet, and of brilliant green in synthetic agar.

		Malachite Green Concentrations									
Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
170	Apr. 17, 1927	12.003	tr	0	0	0	0	0	0	0	0
170	Mar. 25, 1928	—	11.001	—	0	—	0	—	0	—	0
393	Sept. 3, 1927	11.001	11.001	tr	0	0	0	0	0	0	0
393	Apr. 27, 1929	—	11.003	—	0	—	0	—	0	—	0
259	Mar. 12, 1927	11.003	tr	0	0	0	0	0	0	0	0
259	Apr. 17, 1927	12.003	tr	tr	0	0	0	0	0	0	0
259	Mar. 25, 1928	—	11.001	—	0	—	0	—	0	—	0
153	Mar. 11, 1927	11.001	tr	0	tr	0	0	0	0	0	0
153	Apr. 17, 1927	12.006	tr	tr	0	0	0	0	0	0	0
153	Mar. 25, 1928	—	11.001	—	0	—	0	—	0	—	0
210	Apr. 17, 1927	11.001	tr	0	0	0	0	0	0	0	0
210	Mar. 25, 1928	—	11.001	—	0	—	0	—	0	—	0
408	Apr. 27, 1929	—	tr	—	0	—	0	—	0	—	0
411	Apr. 27, 1929	—	tr	—	0	—	0	—	0	—	0
434	Mar. 17, 1928	tr	tr	0	0	0	0	0	0	0	0
434	Apr. 27, 1929	—	tr	—	0	—	0	—	0	—	0
437	Mar. 17, 1928	tr	0	0	0	0	0	0	0	0	0
437	Apr. 27, 1929	—	0	—	0	—	0	—	0	—	0
438	Mar. 17, 1928	tr	tr	0	0	0	0	0	0	0	0
438	Apr. 27, 1929	—	tr	—	tr	—	tr	—	0	—	0

Table 9—Continued.

Crystal Violet Concentrations

Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
170	Mar. 25, 1928	—	11.002	—	11.002	—	11.002	—	11.—	—	tr
170	May 3, 1929	—	11.308	—	11.—	—	tr	—	tr	—	0
393	Aug. 5, 1927	12 012	12.012	11.013	11.013	11 012	11.012	11.012	11.012	11.012	tr
393	Aug. 21, 1927	11 012 11 013	11.012	11.012	11.012	11.012	11.012	11.012	11.012	11.012	11.012
259	Feb. 8, 1927	11.313	11.012 11.013	11.012	tr 11.016	tr	tr 11.013	tr 11.012	tr	tr	tr
259	Mar. 25, 1928	—	11.002	—	11.002	—	11.002	—	tr	—	tr
153	Feb. 8, 1927	11.013	11.013	11.013	11.013	11.013	11.013	11.013	11.013	11.013	tr
153	Mar. 25, 1928	—	11.002	—	11.002	—	11.000	—	tr	—	tr
210	Feb. 8, 1927	11.301	12.302	11.301	11.312	11.312	11.312	11.–11	11.—2	0 tr	11.—1
210	Mar. 25, 1928	—	11.002	—	11.002	—	11.002	—	tr	—	tr
408	July 18, 1927	11.100	11.100	11.100	11.100	tr	tr	tr	tr	tr	tr
408	May 3, 1929	—	11.308	—	tr	—	0	—	0	—	0
411	May 3, 1929	—	11.300	—	11.—	—	tr	—	tr	—	tr
434	Mar. 16, 1928	11.013	11.013	11.013	11.013	11.013	11.013	11.013	tr	tr	tr
434	May 3, 1929	—	11.013	—	11.013	—	11.013	—	tr	—	0
437	Mar. 16, 1928	11.000	11.000	11.000	tr	tr	tr	tr	tr	tr	tr
437	May 3, 1929	—	11.008	—	11.008	—	11.008	—	tr	—	0
438	Mar. 16, 1928	12.001	12 001	11.001	11 001	11.001	11 001	11.001	11.001	11 001	tr
438	May 3, 1929	—	12.003 12.001	—	11.001	—	11.003 11.001	—	11.001	—	tr

Brilliant Green Concentrations

Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
170	July 16, 1927	13.003	12.003	11.003	11.003	0	0	0	0	0	0
393	July 16, 1927	12.213	12.213	11.213	11.213	0	0	0	0	0	0
259	July 16, 1927	13.003	12.206	12.206	11.103	11.103	11.103	tr	tr	tr	tr
153	July 16, 1927	13.003	12.206	11.206	11.106	11.106	11.106	tr	0	0	0
210	July 16, 1927	13.002	11.002	11.002	tr	0	0	0	0	0	0
408	July 16, 1927	12.003	11.000	11.003	tr	tr	0	0	0	0	0

Table 9—Concluded.

Plain Synthetic Agar

170		14.008									
393		—									
259		15 002									
153		15 002									
210		14.008									
408		14.008									
411		14.008									
434		15.008									
437		15 008									
438		15.001									

Copper Sulphate Concentrations

Accession Number	Date of Test	m/1000	m/900	m/800	m/700	m/600	m/500	m/400	m/300	m/200	m/100
210		13 002	13.002	13.002 *	13.002*	12.002*	12.002*	11.002*	tr†	tr†	0

*Greenish color in mycelium as seen from below.
†Halo effect around culture.

Fusarium niveum E. F. Smith (Elegans section)

Cultures used:

M. S. C. 430, received from C. D. Sherbakoff as No. 1016.67, originally isolated from watermelon by H. W. Anderson (Illinois);

M. S. C. 421, received from C. D. Sherbakoff as No. 1016.63, originally isolated from watermelon by H. W. Anderson (Illinois);

M. S. C. 418, received from C. D. Sherbakoff as No. 1016.77, originally isolated from watermelon by H. W. Anderson (Illinois).

This species, as represented by the three organisms used, is very sensitive to malachite green; the growth is white and is restricted in size and stops below midrange. No decolorization of the dye was produced, the edge was usually even but occasionally was recorded as irregular when tufts of mycelium fell down and made the edge uneven. The growth-form was submerged, sericeous, or a combination of the two. Accession 421 in later tests was markedly sensitive to malachite green. On crystal violet, the growth was white, and extended practically through the range of the standard series. The growth was normally 1 or 2 cm. broad in the lower dilutions. No decolorization of the dye, except with organism 418 which decolorized crystal violet strongly even in high concentrations. In the other forms, the mycelium became somewhat blue. All showed a tendency to umbonate growth form, the colony edge was usually even, the growth-form submerged, cottony, or sericeous. Representative readings are given in Table 8. See Plate II.

Fusarium vasinfectum Atk. (Elegans section)

Cultures used:

M. S. C. 176, received February 4, 1924, from G. K. K. Link as No. 1522, originally from Shapavalov as No. 215;

M. S. C. 432, received from C. D. Sherbakoff as No. 1087a, isolated by him from wilted cotton plants;

M. S. C. 433, received from C. D. Sherbakoff;

M. S. C. 439, received from C. D. Sherbakoff as No. 1087b, isolated by him from wilted cotton plants;

M. S. C. 422, received from C. D. Sherbakoff as No. 957.8, isolated by him from wilted cotton plants from South Carolina;

M. S. C. 420, received from C. D. Sherbakoff as No. 1087c, isolated from wilted cotton plants;

M. S. C. 431, received from C. D. Sherbakoff as No. 974a, originally from Fahmy (from India, parasitic on Indian varieties of cotton);

M. S. C. 427, received from C. D. Sherbakoff as No. 1087d, isolated from wilted cotton plants.

On malachite green media, standard series, growth is strongly restricted and limited to the first two steps (1:200,000) aside from slight extensions into the medium in steps three or four, too small to measure ("tr"). Growth is less than 1 cm. broad, white, no decolorization discernible, edge typically even, and the growth-form cottony (11.001). On crystal violet, standard series, the growth is white, and somewhat restricted, though persisting throughout entire range. The growth is

Table 10—Reactions of *Fusarium vasinfectum* and *Fusarium vasinfectum var. inodoratum* on synthetic agar, with and without test dyes.

		Malachite Green Concentrations									
Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
176	Apr. 29, 1928	—	12.002	—	tr	—	0	—	0	—	0
176	Apr. 27, 1929	—	12.013	—	tr	—	0	—	0	—	0
432	Mar. 5, 1929	11.001	tr	tr	0	0	0	0	0	0	0
432	Apr. 27, 1929	—	11.001	—	0	—	0	—	0	—	0
433	Mar. 5, 1929	11.001	tr	tr	0	0	0	0	0	0	0
433	Apr. 27, 1929	—	11.001	—	0	—	0	—	0	—	0
439	Mar. 5, 1929	11.001	tr	tr	0	0	0	0	0	0	0
439	Apr. 27, 1929	—	11.001	—	0	0	0	0	0	0	0
422	Mar. 5, 1929	11.001	11.006 11.000	tr	0	0	0	0	0	0	0
422	Apr. 27, 1929	—	11.001	—	0	—	0	—	0	—	0
420	Mar. 5, 1929	11.001	tr	tr	0	0	0	0	0	0	0
420	Apr. 27, 1929	—	11.001	—	0	—	0	—	0	—	0
431	Mar. 5, 1929	11.001	11.001	tr	0	0	0	0	0	0	0
431	Apr. 27, 1929	—	11.001	—	tr 0	—	0	—	0	—	0
427	Mar. 5, 1929	11.001	11.001	tr	0	0	0	0	0	0	0
427	Apr. 27, 1929	—	11.001	—	0	0	0	0	0	0	0
253*	Apr. 21, 1927	11.001	11.001	tr	0	0	0	0	0	0	0
253*	Apr. 29, 1928	—	11.001	—	tr	—	0	—	0	—	0

*Variety *inodoratum*

Table 10—Continued.

Crystal Violet Concentrations

Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
176	Apr. 29, 1928	—	11.003	—	11.003	—	11.003	—	11.003	—	11.003
176	May 3, 1929	—	12.013	—	11.013	—	11.013	—	11.—1	—	tr
432	Mar. 5, 1929	12.011	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001
432	May 3, 1929	—	11.001	—	11.001	—	11.001	—	11.–01	—	11.—1
433	Mar. 5, 1929	12.011	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001
433	May 3, 1929	—	11.001	—	11.001	—	11.001	—	11.001	—	11.001
439	Mar. 5, 1929	12.011	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001
439	May 3, 1929	—	11.001	—	11.001	—	11.001	—	11.–01	—	11.—1
422	Mar. 5, 1929	12.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001
422	May 3, 1929	—	12.301	—	11.301	—	11.301	—	11.301	—	11.001
420	Mar. 5, 1929	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001
420	May 3, 1929	—	12.201	—	11.201	—	11.201	—	11.201	—	11.001
431	Mar. 5, 1929	12.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001
431	May 3, 1929	—	12.301	—	11.206 11.301	—	11.301	—	11.301	—	11.001
427	Mar. 5, 1929	12.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11.001
427	May 3, 1929	—	12.301	—	11.301	—	11.301	—	11.001	—	11.001
253*	Apr. 29, 1929	—	11.011	—	11.011	—	11.011	—	11.011	—	11.011

*Variety *inodoratum*.

Acriflavine Concentrations

Accession Number	Date of Test	1:10,000	1:5,000	1:3,333	1:2,500	1:2,000	1:1,666	1:1,428	1:1,250	1:1,111	1:1,000
176	Apr. 29, 1928	16.002	—	16.002	—	15.002	—	14.002	—	lost	—

Plain Synthetic Agar

176	14.008
432	14.001
433	14.001
439	14.001
422	14.001
420	14.001
431	14.008
427	14.001

usually about 1 cm. broad, except in the first step which is 1-2 cm. broad; no decolorization occurred with possible exception of weak decolorization with Accession 420 and some color change by Accessions 422, 431, and 427 in which the center of the mycelial mat, as seen from beneath, became blue; the edge is typically even; and the growth-form cottony. On crystal violet, with these organisms, a tendency to lavender color in the mycelium and to umbonate growth was noted. On acriflavine, strong growth, approximating the check in most concentrations, with villous mycelium. Representative readings are given in Table 10. On plain synthetic agar, the mycelium was white, about 4-5 cm. broad, even edged, and cottony or woolly in growth-form. See Plate IV.

Fusarium vasinfectum Atk. var. *inodoratum* Wr. (Elegans section)

Culture used:

M. S. C. 253, received from H. W. Wollenweber, July, 1924, as No. 1556.

This variety, represented by the single culture, M. S. C. 253, was sensitive to malachite green, growth in the standard series being limited to the first or second steps, with slight growth ("tr") persisting somewhat further. The growth on the second step in one test was white, less than 1 cm. broad, producing no decolorization of the dye, the edge was even, and the growth-form cottony (11.001). On crystal violet, standard series, restricted growth occurred in all dilutions to the eighth step and possibly throughout the range (1 test). The growth was white, .5 cm. to 1 cm. broad, and produced no change in medium. The edge was typically uneven, and the growth-form typically cottony (11.011) (Table 10).

It would be difficult with the dye media to separate this organism from *Fusarium conglutinans* and its allies unless advantage were taken of the fact that *Fusarium conglutinans* and its varieties are not known to produce color with any natural medium such as rice.

Gibberella saubinetii (Mont.) Sacc. (Saubinetii section)
(*Fusarium graminearum* Schwabe)

Cultures used:

M. S. C. 221, received from C. D. Sherbakoff, April 20, 1924, as No. 827a and determined by him;

M. S. C. 183, received from E. C. Stakman, February 8, 1924, as No. 4 (under name *F. culmorum*), collected at University Farm, St. Paul, Minnesota.

This species, as represented by the two accessions used, gave restricted growth on the standard malachite green series, usually limited to first two steps (1:200,000), but occasionally persisting as a "trace" in higher concentrations. Growth white, from .5 to 1 cm. broad, producing no decolorization of dye, strongly frondose, and typically cottony in growth-form. On the standard crystal violet series, the organism grew throughout and slightly beyond the range, with restricted growth throughout, about 1 to 2 cm. broad in the lower half of the range, then .5 to 1 cm. A typical reading for the organism at midrange is 11.002 (Table 11). On plain synthetic agar, the growth was strong and of villous growth-form (14.-02).

Table 11—Representative reactions of various Fusaria of 'A, Group 3' to concentrations of malachite green and crystal violet in synthetic agar.

Malachite Green Concentrations

Species	Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
Gibberella saubinetii	221	Mar. 10, 1927....	11.021	0 / tr	0	tr	0	0	0	0	0	0
Gibberella saubinetii	221	Apr. 27, 1929....	—	11.021	—	0	—	0	—	0	—	0
Gibberella saubinetii	183	Mar. 10, 1927....	11.003	tr	tr	0	tr	0	0	0	0	0
Gibberella saubinetii	183	Apr. 29, 1928....	—	tr	—	0	—	0	—	0	—	0
F. conglutinans.....	156	Apr. 29, 1928....	—	11.006	—	0	—	0	—	0	—	0
F. conglutinans.....	156	Apr. 20, 1929....	11.003	11.003	—	0	—	0	—	0	—	0
F. conglutinans.....	165	Apr. 29, 1928....	—	11.006	—	tr	—	0	—	0	—	0
F. conglutinans.....	165	Apr. 20, 1929....	11.003	11.002	—	0	—	0	—	0	—	0
F. viride.....	312	Apr. 27, 1929....	—	11.013	—	0	—	0	—	0	—	0
F. striatum.....	320	Apr. 14, 1929....	—	—	11.003	—	0	—	0	—	0	—

Crystal Violet Concentrations

Species	Accession Number	Date of Test	1:20,000	1:10,000	1:6,666	1:5,000	1:4,000	1:3,333	1:2,857	1:2,500	1:2,222	1:2,000
Gibberella saubinetii	221	Mar. 19, 1929....	11.003	11.006	11.002	11.002	11.002	11.002	tr	0	0	0
F. conglutinans.....	156	Mar. 19, 1929....	12.006	11.006	11.006	11.006	11.006	11.006	tr	tr	tr	tr
F. conglutinans.....	165	Mar. 19, 1929....	12.006	11.006	11.006	11.006	11.006	11.006	tr	tr	tr	tr
F. viride.....	312	Mar. 19, 1929....	12.006	11.013	11.013 / 11.000	11.013	11.000 / 11.013	11.013	tr	11.013	11.013	11.013
F. striatum.....	320	Mar. 19, 1929....	12.206	11.213	11.213	11.213	11.213	11.213	11.213	11.213	11.213	11.213

Fusarium conglutinans Wr. (Elegans section)

Cultures used:

M. S. C. 156, received from L. R. Jones as Wisconsin 251.
M. S. C. 165, received from G. K. K. Link as No. 1594, originally from L. R. Jones as No. 175.

On the standard malachite green series, mycelium was white, restricted in growth, less than 1 cm. broad, with growth stopping at third or fourth step (1:66,666). No decolorization discernible, edge of colony usually even, growth-form sericeous or submerged with sericeous center. On the standard crystal violet series, growth extended throughout the range and one or two steps beyond (1:3,333), restricted in the lower dilutions to 1-2 cm. broad, then less than 1 cm. broad in the stronger concentrations. No decolorization of the dye was produced, the edge was usually even except as wisps of sericeous threads falling made the edge irregular. The growth-form was sericeous or submerged with sericeous center. Both organisms grew throughout the acriflavine series used (Table 11).

Culture M. S. C. 156 was grown in a graded series of 10 in which copper sulphate was added to give molar concentrations from m/1000 to m/100. Growth occurred in measurable amount in medium containing copper sulphate m/400 (11.001). The weaker concentrations m/500-m/1000 showed the same growth. This tolerance of fairly high concentrations of copper sulphate may be of value in differentiating this species.

On the plain synthetic agar, the growth was strong, circular in outline, and submerged, or occasionally villous in form. See plate V.

Fusarium viride (Lehm.) Wr. (Martiella section)

Culture used:

M. S. C. 312, received from C. L. Shear as No. 905, isolated from soil, Honduras.

On malachite green media, the growth was white, less than 1 cm. broad in the second step of the standard series (1:200,000), no growth occurring in the fourth step. No decolorization was produced, the edge was ramose, and the growth-form sericeous. On the standard crystal violet media, the organism proved extremely resistant to the dye, growth occurring throughout the standard series 1 to 2.5 cm. broad depending on the concentration, and with the extended series measurable growth was produced in a concentration dye of 1:2,000. At this strong concentration, the organism was white, between .5 and 1 cm. broad, produced no decolorization, the edge was ramose, and the growth-form sericeous (Table 11). On plain synthetic agar, the reading was 14.013.

Fusarium striatum Sherb. (Martiella section)

Cultures used:

M. S. C. 320, received from C. L. Shear as No. 921, originally from International Bureau of Fungous Cultures, Holland;
M. S. C. 314, received from C. L. Shear as No. 907, isolated from papaya fruit, Honduras.

On the standard malachite green series, growth was strongly restricted and was checked at about the second or third step, (1:100,000). In weak concentrations, in early tests, malachite green was decolorized slightly. The growth in the second step (1:200,000) with Accession 320 was less than 1 cm. broad, no decolorization was discernible, the edge was even, and growth-form sericeous. Accession 314, grew only slightly in malachite green. Dr. C. D. Sherbakoff, on the basis of morphological examination, questioned the assignment of this accession to this species. On crystal violet, Accession 320 grew throughout the range, with little evidence in the lower concentrations of toxic effects. On the extended range, both strains used grew throughout, withstanding a concentration of the dye of 1:2,000. Both Accessions decolorized crystal violet strongly, the edge of the growth was ramose and the growth-form cottony or submerged with cottony or sericeous center. In the 1:2,000 concentration, the readings were for Accession 314, 12.201 and for Accession 320, 11.213. On plain synthetic agar, the readings were as follows: Accession 314, 15.005; Accession 320, 15.000, 14.002, and 14.000.

A, Group 4.

Fusarium acuminatum E. and E. em. Wr. (Gibbosum section)

Culture used:

M. S. C. 283, received from C. L. Shear as No. 859, isolated in Honduras.

On malachite green, standard series, growth was somewhat restricted and limited to the first two steps (1:200,000). The mycelium was white, 1-2 cm. broad in the first and second steps and less than .5 cm. in 1:100,000 concentration. Decolorization of the dye was produced and the edge of the colony was somewhat irregular; the growth-form was either sericeous or cottony. On crystal violet, standard series, no growth occurred (Table 12). On synthetic agar, the organism was white, 4 cm. broad, edge even, and submerged. The growth-form showed distinctive revolute type similar to a coiled plume, which may be of enough constancy to identify the species.

Fusarium lini Bolley (Elegans section)

Culture used:

M. S. C. 189, received from E. C. Stakman as No. 10.

On malachite green, standard series, fairly strong growth (3 cm.) occurred on the first step (1:400,000), 1-2 cm. on the second step, and 1 cm. on the third step (1:100,000). No growth occurred in higher concentrations. No decolorization was produced, the edge was uneven, and the growth-form sericeous. On crystal violet, standard series, the growth was white, very restricted in second step (1:200,000) and only as a trace in the 1:100,000 concentration. No decolorization was evident, edge even, and growth-form cottony (11.001). In general, this organism grew strongly, usually submerged, seems to be characterized by considerable tolerance of malachite green and lack of tolerance of crystal violet (Table 12). On plain synthetic agar, the organism grew strongly, usually submerged (14.000).

Table 12—Representative reactions of various Fusaria of 'A, Group 4' to malachite green and crystal violet in synthetic agar.

Malachite Green Concentrations

Species	Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
F. acuminatum	283	May 3, 1925	12.213	12.213	0 tr	0	0	0	0	0	0	0
F. lini	189	Apr. 27, 1929	—	12.013	—*	0	—	0	—	0	—	0
F. zonatum	322	Apr. 27, 1929	—	12.013	—*	0	—	0	—	0	—	0
F. sclerotioides	168	May 3, 1925	14.206	12.200 12.206	tr	0	0	0	0	0	0	0
F. caudatum	290	Apr. 27, 1929	—	11.001	—	0	—	0	—	0	—	0
F. batatatis	226	Apr. 21, 1927	13.003	12 003	11.006	0	0	0	0	0	0	0
F. batatatis	169	Apr. 21, 1927	12.001	11.001	11.001	tr	0	0	0	0	0	0
F. radicicola	202	Apr. 17, 1927	14.001	12.003	tr	0	0	0	0	0	0	0
F. radicicola	203	Mar. 10, 1927	12.013	12.013	0	0	0	0	0	0	0	0

Crystal Violet Concentrations, Standard Series

Species	Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
F. acuminatum	283	May 3, 1929	—	0	—	0	—	0	—	0	—	0
F. lini	189	May 3, 1929	—	11.001	—	11.—	—	tr	—	0	—	0
F. zonatum	322	May 3, 1929	—	11.008	—	11.008	—	11.008	—	tr	—	0
F. sclerotioides	168	May 3, 1929	—	11.008	—	11.013	—	11.008	—	tr	—	0
F. caudatum	290	Feb. 8, 1927	12.013	11.013	11.013	11.013	0	0	tr 0	tr	0	tr
F. batatatis	226	Feb. 8, 1927	12.113	11 113	11.113	11 113	11.013	11.013	11 013	tr 11.013	tr 11.013	tr
F. batatatis	169	Feb. 8, 1927	12 002	12.002	11.102	11.102	11.102	tr	11 102	11.102	tr	11 102

Crystal Violet Concentrations, Extended Series

Species	Accession Number	Date of Test	1:20,000	1:10,000	1:6,666	1:5,000	1:4,000	1:3,333	1:2,857	1:2,500	1:2,222	1:2,000
F. radicicola	202	Apr. 19, 1929	12.202	11 202	11.202	11.202	11.202	11.202	11 202	tr	tr	tr
F. radicicola	203	Apr. 19, 1929	12 202	11 202	11 202	11.202	11.202	11.202	11.213	tr	tr	tr

*1 cm. broad in this concentration in other tests.

Fusarium hyperoxysporum Wr. (Elegans section)

Cultures used:

M. S. C. 162, received from G. K. K. Link, February 4, 1924, as No. 1593, originally L. L. Harter's No. 4058a;

M. S. C. 225, obtained from C. D. Sherbakoff, April 20, 1924, as No. 887f, originally from L. L. Harter's No. 4068a;

M. S. C. 270, received from H. W. Wollenweber, July 19, 1924, as No. 3756;

M. S. C. 332, received from C. L. Shear as "*F. lutulatum* Sherb.", No. 944.

The following general statement includes with but slight exception the reactions of these cultures: On malachite green, standard series, the growth of the mycelial mat was restricted even in the lowest concentrations. The size of the mat was from 1-2 cm. in the lowest concentration (1:400,000) and 0.5 to 1 cm. in the 1:200,000 concentration of dye. The growth was white and produced no decolorization. The edge was typically even. In certain cases where sericeous growth occurred, the edge was irregular due to falling over of the tufts. The typical growth-form was submerged with sericeous center.

On crystal violet, the growth was restricted even in the lowest concentration (1:40,000), being less than 1 cm. in width. Growth occurred only in lowest concentrations (1:40,000 and 1:20,000), except for extremely slight growth consisting of faint wisps of mycelium pushing out from the inoculum which were too small to measure. Color was intensified somewhat in the mycelium. The edge of the growth was even, and the growth-form was submerged or submerged with sericeous tufts in the center.

On brilliant green, standard series, there was a moderately strong growth in the lower half of the range, becoming checked at about the 1:25,000 concentration. Strong decolorization occurred throughout; the edge was slightly irregular, and the growth-form was sericeous or submerged with sericeous center.

On acriflavine, standard series, the reaction was similar in all strains, growth occurring throughout the range, growth being either submerged or villous.

On plain synthetic agar, the organism grew strongly (4-5 cm. broad), submerged, weakly cottony or with sericeous center. The detailed record of representative reactions is given in Table 13. See Plate VI.

Fusarium zonatum Sherb. (Elegans section)

Culture used:

M. S. C. 322, received by H. W. Wollenweber from International Bureau of Fungous Cultures, Holland, obtained from C. L. Shear, December 8, 1924, as No. 924.

On malachite green, standard series, the organism used grew on the second step (1:200,000) but not on the fourth. The size of mycelium growth as recorded in one test was 2 cm.; no decolorization was observed, edge irregular, growth-form sericeous (12.013). On crystal violet, the growth was restricted, less than 1 cm. broad, extending to about sixth or seventh step.

Table 13—Results with various strains of *Fusarium hyperoxysporum* on synthetic media, with and without triphenylmethane dyes.

Malachite Green Concentrations

Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
162	Apr. 3, 1927	12.006	tr	tr	0	0	0	0	0	0	0
162	Mar. 6, 1928	—	11.006	—	tr	—	0	—	0	—	0
225	Apr. 3, 1927	12.006	tr	tr	0	0	0	0	0	0	0
225	Mar. 6, 1928	—	11.006	—	tr	—	0	—	0	—	0
270	Mar. 12, 1927	12.006	11.006	11.006	tr	tr	tr	0	0	0	0
270	Apr. 3, 1927	12.006	tr	0	0	0	0	0	0	0	0
270	Mar. 6, 1928	—	11.006	—	tr	—	0	—	0	—	0
332	Apr. 3, 1927	13.006	tr	tr	0	0	0	0	0	0	0
332	Mar. 6, 1928	—	11.003	—	0	—	0	—	0	—	0

Crystal Violet Concentrations

Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
162	Apr. 13, 1927	11.300	11.300	tr	tr	tr	tr	tr	tr	—	tr
162	Mar. 6, 1928	—	11.006	—	tr	—	tr	—	tr	—	tr
225	Apr. 13, 1927	11.300	11.300	tr	tr	tr	tr	tr	tr	tr	tr
225	Mar. 6, 1928	—	11.006	—	tr	—	tr	—	tr	—	tr
270	Apr. 13, 1927	11.306	11.306	tr	tr	tr	tr	tr	tr	tr	tr
270	Mar. 6, 1928	—	11.003	—	tr	—	tr	—	tr	—	tr
332	Apr. 13, 1927	11.306	11.306	tr	tr	tr	tr	tr	tr	tr	tr
332	Mar. 6, 1928	—	11.013	—	tr	—	tr	—	tr	—	tr

Table 13—Continued.

Brilliant Green Concentrations

Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
162	May 15, 1927	13.206	13.206	12.213	12.213	11.213	11.213	tr	tr	tr	0
225	May 15, 1927	13.206	13.206	12.213	12.213	11.213	11.213	tr	tr	tr	0
270	May 15, 1927	13.206	13.206	12.213	12.213	12.213	12.213	11.213	11.213	11.213	tr
332	May 15, 1927	13.206	13.206	12.213	12.213	12.213	12.213	11.213	11.213	11.213	0

Acriflavine Concentrations

Accession Number	Date of Test	1:10,000	1:5,000	1:3,333	1:2,500	1:2,000	1:1,666	1:1,428	1:1,250	1:1,111	1:1,000
162	Mar. 6, 1928	15.000	—	14.002	—	13.002	—	12.002	—	11.002	—
225	Mar. 6, 1928	15.000	—	14.002	—	13.002	—	12.002	—	11.002	—
270	Mar. 6, 1928	15.000	—	14.002	—	13.002	—	12.001	—	tr	—
332	Mar. 6, 1928	15.000	—	14.002	—	13.002	—	12.002	—	11.002	—

Plain Synthetic Agar

162		15.000									
225		15.000									
270		15.001									
332		15.001									

No decolorization was produced, the edge was even, and the growth-form woolly. (11.008.) See Table 12. On plain synthetic agar, the growth was normal size (3-4 cm.), circular, and woolly (14.-08).

Fusarium sclerotioides Sherb. (Elegans section)

Culture used:

M. S. C. 168, received from G. K. K. Link as No. 1631; originally from H. W. Wollenweber as No. 3757.

On synthetic medium containing malachite green, standard series, the growth was white, about 2 cm. broad in 1:200,000 dilution and limited to the lowest quarter of the range of dilutions. The malachite green was usually decolorized. The edge was even and the growth-form either submerged with cottony (or sericeous) center or entirely submerged in some tests. On medium containing crystal violet, standard series, the growth was white, 1 cm. broad, and checked at about the sixth or seventh step. No decolorization or change in the dye occurred, the edge was typically even and the growth-form woolly to sericeous. (Table 12.) On plain synthetic agar, the growth was strong, 4 cm. broad, submerged or woolly.

Fusarium caudatum Wr. (Gibbosum section)

Culture used:

M. S. C. 290, received from C. L. Shear as No. 870, originally isolated from soil, Jamaica.

This organism usually grew with somewhat restricted growth in the lowest quarter of the standard malachite green series, the breadth of the mycelial mat in the 1:200,000 dilution being about 1 cm. or slightly more. The growth produced no decolorization of the dye, had an even edge, and was typically cottony. On the standard crystal violet series, this species, as represented by the single culture available, grew with somewhat restricted growth up to the fifth step (1:8,000). The mycelium was white, 1-2 cm. broad in the lowest concentration, then .5 to 1 cm.; no decolorization was produced, the edge was irregular and the growth-form sericeous (11.013). See Table 12. On plain synthetic agar the organism was white, 4 cm. broad, and submerged (14.-00).

Fusarium orthoceras App. & Wr. (Elegans section)

Culture used:

M. S. C. 158, received from G. K. K. Link as No. 1629, originally from H. W. Wollenweber as No. 974.

On malachite green, standard series, the growth was white, 2 cm. broad in lowest concentration (1:400,000), growth stopping sharply at about the third step (1:100,000). No decolorization was observed, the edge was often uneven, and the growth-form sericeous. On crystal violet, standard series, the growth was white, 2 cm. broad in lowest concentration, stopping at about the middle of the range (1:10,000), although in some cases persisting as a very restricted growth throughout the range. No decolorization noted, the edge was even, growth-form typically submerged with sericeous center. On brilliant green, standard series, the growth was white, 4 cm. broad in lowest

concentration, reducing to 1-2 cm. breadth at about 1:40,000 concentration, and then a trace in the highest concentrations. No decolorization noted, the edge was even, and the growth-form typically submerged with sericeous center. See Table 14. On acriflavine, standard series, fairly strong growth (2 cm.) occurred in the highest concentration used (1:1,000). On plain synthetic agar, there was typically submerged, even-edged growth, 3-4 cm. broad.

Accession No. 258, received from H. W. Wollenweber as No. 1247, *F. orthoceras*, gave similar readings but was not included because tests on certain dyes had not been carried on. Accession No. 188, received as *F. orthoceras* var. *triseptatum* from E. C. Stakman, originally Edson's No. 9, isolated from a rotted potato tuber, was not accepted as typical by C. D. Sherbakoff. See Plate II.

Fusarium orthoceras App. & Wr. var. *triseptatum* Wr. (Elegans section)

Culture used:

M. S. C. 277, received from C. L. Shear as No. 899, December, 1924; originally isolated from banana trash, Tela, Honduras.

This organism showed reactions on malachite green similar to those recorded for *Fusarium orthoceras* 158, except the growth-form was submerged with woolly center. On crystal violet, growth reactions were similar to Accession 158, except that the growth-form was cottony. It was slightly more sensitive to brilliant green than *Fusarium orthoceras* 158.

Fusarium orthoceras App. & Wr. var. *longius* Sherb. (Elegans section)

Culture used:

M. S. C. 159, received from G. K. K. Link, February 4, 1924, as No. 1636, originally from H. W. Wollenweber as No. 3752.

The reactions of this variety to malachite green, standard series, were very similar to preceding accessions. On crystal violet, extended series, growth persisted through 1:2,500 concentration typically .5 to 1 cm. broad. No decolorization was produced. The edge was even and the growth-form cottony (11.001). See Table 14. On brilliant green and acriflavine, the reactions were very similar to those recorded for Accessions 158 and 277. On plain synthetic agar, the growth was strong and of villous character.

Fusarium oxysporum Schlecht. (Elegans section)

Cultures used:

M. S. C. 160, received from G. K. K. Link, February 4, 1924; as No. 1505, originally from Shapavalov as No. 207;

M. S. C. 209, received from R. W. Goss, as No. 43, originally from H. W. Wollenweber;

M. S. C. 309, received from C. L. Shear as No. 900, isolated in Honduras;

M. S. C. 211, received from L. R. Jones as Wisc. No. 227, obtained by Wisconsin laboratory from G. K. K. Link as Harter No. 3395.[1] Apparently same as M. S. C. 204, and M. S. C. 55;

M. S. C. 204, received from R. W. Goss as No. 42, or Wisc. 227, obtained from G. K. K. Link as No. 3395.[1] Apparently same as M. S. C. 211 and M. S. C. 55;

[1]See footnote page 48

Table 14—Reactions of *Fusarium orthoceras* and its varieties to various concentrations of malachite green, of crystal violet, and of brilliant green in synthetic agar and to plain synthetic agar.

Malachite Green Concentrations

Species	Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
F. orthoceras	158	Mar. 10, 1927	12.013	12.013	tr	tr	0	0	0	0	0	0
F. orthoceras	158	Mar. 26, 1928	—	11.003	—	tr	—	0	—	0	—	0
F. orthoceras	158	Apr. 27, 1929	—	12.013	—	0	—	0	—	0	—	0
F. orthoceras var. triseptatum	277	Apr. 17, 1927	12.001	12.005	11.005	tr	0	0	0	0	0	0
F. orthoceras var. triseptatum	277	Mar. 26, 1928	—	11.001	—	tr	—	0	—	0	—	0
F. orthoceras var. triseptatum	277	Apr. 27, 1929	—	12.008	—	0	—	0	—	0	—	0
F. orthoceras var. longius	159	Mar. 10, 1927	12.001	11.001	tr	0	0	0	0	0	0	0
F. orthoceras var. longius	159	Apr. 17, 1927	12.001	11.001	tr	tr	0	0	0	0	0	0
F. orthoceras var. longius	159	Mar. 26, 1928	—	11.003	—	tr	—	0	—	0	—	0

Crystal Violet Concentrations

Species	Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
F. orthoceras	158	May 3, 1927	11.006	11.006	11.006	11.006	tr	tr	tr	tr	tr	tr
F. orthoceras	158	Mar. 26, 1928	—	11.002	—	11.002	—	11.002	—	11.002	—	11.001
F. orthoceras	158	Mar. 19, 1929	—	12.006	—	11.006	—	tr	—	tr	—	tr
F. orthoceras var. triseptatum	277	May 13, 1927	11.001	11.001	tr	11.001	tr	tr	tr	tr	tr	tr
F. orthoceras var. triseptatum	277	Mar. 26, 1928	—	11.001	—	11.001	—	11.001	—	11.001	—	11.001
F. orthoceras var. longius	159	May 3, 1927	11.001	11.001	11.001	11.001	11.001	11.001	11.001	11 001	11.001	11.001
F. orthoceras var. longius	159	Mar. 26, 1928	—	11.001	—	11.012	—	11.013	—	11.013	—	11.013
F. orthoceras var. longius	159	Mar. 19, 1929	—	11.013	—	11.001	—	11.001	—	11.001	—	11.001*

*Growth, with same reading, continued to 1:2,500 concentration and as "tr" up to 1:2,000 concentration.

Table 14—Continued.

Brilliant Green Concentrations

Species	Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
F. orthoceras	158	July 3, 1927	14.000	13.006	13.006	12.006	12.106	12.106	tr	tr	tr	0
F. orthoceras var. triseptatum	277	July 3, 1927	13.000	13.002	12.002	12.002	11.102	11.102	tr	tr	tr	0
F. orthoceras var. longius	159	July 3, 1927	13.000	12.002	12.002	12.001	11.101	11.101	0	0	0	0

Plain Synthetic Agar

F. orthoceras	158		14.000									
F. orthoceras var. triseptatum	277		14.008									
F. orthoceras var. longius	159		15.002									

M. S. C. 55, received from Washington as No. 3395,[1] about 1913. Maintained in stock culture since then at Michigan State College,

M. S. C. 212, received from L. R. Jones as Wisc. 382, originally from H. W. Wollenweber;

M. S. C. 208, received from R. W. Goss as No. 39, originally from MacMillan as Washington No. 1467;

M. S. C. 321, received from C. L. Shear as No. 922, originally from International Bureau of Fungous Culture, Holland, through courtesy of H. W. Wollenweber;

M. S. C. 185, received from E. C. Stakman, February 8, 1924, as No. 6, isolated from potato by G. R. Bisby;

M. S. C. 310, received from C. L. Shear as No. 901, "*Fusarium oxysporum* Schlecht. var. *nicotianae* John."

On malachite green, standard series, the mycelial growth was white, usually somewhat restricted (2 cm.) in the lowest concentration (malachite green concentration 1:400,000) and definitely stopped at the second or third step (1:100,000), except for very slight extrusions of hyphae too small to measure which occasionally occurred in higher concentrations. No change in the medium discernible. The edge was typically even, but when the growth-form was sericeous the edge might be irregular due to falling of clumps of threads. The growth-form was somewhat variable with tendency to aerial growth throughout, probably the sericeous type or the submerged with sericeous center was most common. Strong variation in the character of aerial mycelium was found in the different readings.

On crystal violet, standard series, the growth was white and strongly restricted (.5 to 1 cm., rarely 2 cm.) but persisting throughout the range or practically so (1:5,000 or 1:4,000). No decolorization of the dye was produced. The edge was even and the growth-form, typically, cottony. On brilliant green, the growth persisted to the eighth or ninth step (1:25,000), gradually decreasing from approximately 3 cm. breadth to a small clump about .05 cm. across. The edge was prevailingly even, except where sericeus growth occurred and the falling wefts gave the growth an uneven edge. On brilliant green, a characteristic, strong decolorization of the dye was produced in practically every case. On acriflavine, growth occurred practically throughout the range, diminishing gradually from 4-5 cm. breadth to 1 cm. as the concentration increased from 1:10,000 to 1:1,000. On synthetic media, containing copper sulphate, the toxic limit was reached when the media contained copper sulphate in about concentration m/300. Culture M. S. C. 309 in the test grew in media containing m/200 copper sulphate and produced around the mycelial mat a strong halo effect. See Table 15. The growth-form was submerged in practically all cases, except for culture M. S. C. 309, which had cottony, aerial mycelium throughout.

In general, it is concluded that the various organisms selected as representative of the species *Fusarium oxysporum* responded with great constancy to the various toxic agents, if allowance is made for unavoidable differences in technic which call forth minor variations. Certain varieties commonly recognized as distinct, recognizable entities such as *F. oxysporum* var. *nicotianae*, seem to fall within the general scope of the species as outlined. The

[1] *F. oxysporum*, Washington 3395, is a reisolation of Washington 2413 isolated and identified by H. W. Wollenweber in January, 1913, cf. C. W. Carpenter, Potato tuber rots caused by Fusarium spp. Journ. Agr. Res. 5: 183-209, illus. 1915.

Table 15—Reactions of various accessions of *Fusarium oxysporum* and of *Fusarium oxysporum* var. *nicotianae* to various concentrations of triphenylmethane dyes and of copper sulphate in synthetic agar.

		Malachite Green Concentrations									
Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
160	Mar. 10, 1927	12.000 12.006	11.002	tr	tr	tr	tr	0	0	0	0
160	Mar. 6, 1928	—	12.000	—	tr	—	0	—	0	—	0
160	Apr. 27, 1929	—	12.000	—	0	—	0	—	0	—	0
209	Mar. 10, 1927	12.002	11.—	tr	0	0	0	0	0	0	0
209	Mar. 6, 1928	—	12.001	—	tr	—	0	—	0	—	0
309	Mar. 10, 1927	11.–02	11.–02	0	0	0	0	0	0	0	0
309	Apr. 3, 1927	12.003	tr	tr	0	0	0	0	0	0	0
309	Mar. 6, 1928	—	12.003	—	tr	—	0	—	0	—	0
211	Apr. 3, 1927	12.001	tr	tr	0	0	0	0	0	0	0
211	Mar. 6, 1928	—	12.001	—	tr	—	0	—	0	—	0
204	Mar. 10, 1927	13.006	12.006	11.006	tr	tr	0	0	0	0	0
204	Apr. 3, 1927	12.006	tr	tr	0	0	0	0	0	0	0
204	Mar. 6, 1928	—	12.003	—	tr	—	0	—	0	—	0
204	Apr. 27, 1929	—	12.006	—	0	—	0	—	0	—	0
55	Mar. 10, 1927	12.003	11.003	tr	0	0	0	0	0	0	0
55	Apr. 3, 1927	12.003	tr	0	0	0	0	0	0	0	0
55	Mar. 6, 1928	—	12.003	—	tr	—	0	—	0	—	0
212	Mar. 10, 1927	13.006	12.006	tr	tr	tr	tr	0	0	0	0
212	Apr. 3, 1927	12.003	tr	0	0	0	0	0	0	0	0
212	Mar. 6, 1928	—	12.003	—	tr	—	0	—	0	—	0
208	Mar. 10, 1927	11.005	11.—	tr	0	0	0	0	0	0	0
208	Apr. 3, 1927	12.002	tr	tr	0	0	0	0	0	0	0
208	Mar. 6, 1928	—	12.003	—	tr	—	0	—	0	—	0
321	Mar. 10, 1927	12.006	11.002	tr	tr	0	0	0	0	0	0
321	Apr. 3, 1927	12.003	tr	tr	0	0	0	0	0	0	0
321	Mar. 6, 1928	—	12.003	—	tr	—	0	—	0	—	0
321	Apr. 17, 1929	—	12.006	—	0	—	0	—	0	—	0
185	Apr. 3, 1927	12.006	tr	0	0	0	0	0	0	0	0
185	Mar. 6, 1928	—	12.006	—	tr	—	0	—	0	—	0
310*	Mar. 10, 1927	12.013	tr	tr	0	0	0	0	0	0	0
310*	Mar. 6, 1928	—	12.006	—	tr	—	0	—	0	—	0
310*	Apr. 17, 1929	—	12.006	—	11.003	—	0	—	0	—	0

*Var. *nicotianae*.

Table 15—Continued.

Crystal Violet Concentrations

Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
160	May 5, 1929	—	11.001	—	11.001	—	tr	—	0	—	tr
209	May 5, 1929	—	11.001	—	11.001	—	11.001	—	11.001	—	tr
309	May 5, 1929	—	11.001	—	11.001	—	11.001	—	11.001	—	tr
211	May 5, 1929	—	—	—	—	—	—	—	—	—	—
204	May 5, 1929	—	11.001	—	11.001	—	11.001	—	11.001	—	tr
55	May 5, 1929	—	—	—	—	—	—	—	—	—	—
212	May 5, 1929	—	12.013	—	11.013	—	11.001	—	11.001	—	tr
208	May 5, 1929	—	11.001	—	11.001	—	11.001	—	11.001	—	tr
321	May 5, 1929	—	11.001	—	11.001	—	tr	—	11.001	—	tr
185	May 5, 1929	—	—	—	—	—	—	—	—	—	—
310*	May 5, 1929	—	11.013	—	11.003	—	11.—	—	tr	—	tr
204	Mar. 6, 1928	—	11.001	—	11.001	—	11.001	—	11.001	—	11.001
211	Mar. 6, 1928	—	11.001	—	11.001	—	11.001	—	11.001	—	11.001
55	Mar. 6, 1928	—	11.001	—	11.001	—	11.001	—	11.001	—	11.001
185	Mar. 6, 1928	—	11.001	—	11.001	—	11.001	—	11.001	—	11.001
160	Mar. 6, 1928	—	11.001	—	11.001	—	11.001	—	11.001	—	11.001
310*	Mar. 6, 1928	—	11.000	—	11.000	—	11.001	—	tr	—	tr

*Var. *nicotianae*.

Brilliant Green Concentrations

Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
160	May 5, 1927	13.206	13.201	13.201	12.201	12.201	11.201	11.201	tr	tr	tr
209	May 5, 1927	13.001	13.001	12.201	12.001	12.201	11.201	11.201	tr	tr	0
309	May 5, 1927	13.201	13.201	12.201	12.201	11.201	11.201	11.201	11.201	tr	0
211	May 5, 1927	13.201	13.201	12.201	12.201	12.201	11.201	11.201	tr	tr	tr
204	May 5, 1927	13.206	13.206	12.213	12.213	12.203	11.213	11.213	11.213	tr	0
55	May 5, 1927	13.206	13.206	13.206	12.213	11.213	11.213	11.213	0	0	0
212	May 5, 1927	13.206	13.206	13.213	12.213	11.213	11.213	tr	tr	tr	tr
208	May 5, 1927	13.206	13.206	12.213	12.213	12.213	11.213	tr	tr	tr	tr
321	May 5, 1927	13.206	13.206	12.213	12.213	11.213	11.213	tr	tr	tr	0
185	May 5, 1927	13.206	13.213	13.213	12.213	11.213	11.213	11.213	tr	tr	tr
310*	May 5, 1927	13.206	13.206	12.213	12.213	11.213	11.213	tr	tr	tr	tr

*Var. *nicotianae*.

Table 15—Concluded.

Copper Sulphate Concentrations

Accession Number	Date of Test	m/1000	m/900	m/800	m/700	m/600	m/500	m/400	m/300	m/200	m/100
160	Jan. 2, 1929	14.001	13.000	13.000	12.000	12 000	12 000	11.000	11.000	tr	0
209	Jan. 2, 1929	12.000	12.000	12.000	12.000	12.000	12.000	11 000	tr	0	0
309	Jan. 2, 1929	15.001	15.001	15.001	14.001	14 001	13 001	13 001	13 001	12.001	0
211	Jan. 2, 1929	12.000	12.000	12.000	12.000	12 000	12.000	11.000	tr	0	0
204	Jan. 2, 1929	13.006	13.006	13.006	12 006	12 000	12.000	11.000	11.000	0	0
55	Jan. 2, 1929	14.000	13.000	13.000	12 000	12 000	12 000	11 000	11.000	0	0
212	Jan. 2, 1929	12 000	12.000	—	12.000	12.001 12 000	12.000	11.000	tr	0	0
208	Jan. 2, 1929	12.000	12.100 12.000	12.000	12.000	12.000	12.000	11 000	tr	0	0
321	Jan. 2, 1929	14.000	12.000	12.000	12.000	12.000	11.000	tr	12 001 12 000	0	0
185	Jan. 2, 1929	13.000	13.000 12.000	13.000	12.000	12 000	12 000	11 000	11.000	0	0
310	Jan. 2, 1929	12.000	12.000	12.002	12.000	12.000	12.000	11.000	tr	0	0

striking thing to be noticed for the *F. oxysporum*, as with a few related groups, is that crystal violet seems uniformly to have restricted the size of growth more than malachite green, although malachite green limited the growth to the first fifth or quarter of the standard range. The growth persisted almost throughout the range of crystal violet used. The decolorization of brilliant green was constant and is in strong contrast to the absence of such decolorization with the *Fusarium orthoceras* accessions used.

Attention is called particularly to Accessions 211, 204, and 55 which, from their record, originated from the same isolation, No. 3395, distributed from the Office of Cotton, Truck, and Forage Crop Diseases. These accessions after their varied history, were found by C. D. Sherbakoff upon examination in August, 1929, to be typical *Fusarium oxysporum*. Their response to the toxic dyes was closely watched in numerous tests and they did not vary much in their salient reactions. The reactions chosen for Table 15 are the extremes of the readings made. See Plates VI and VII.

Fusarium batatatis Wollenweber (Elegans section)

Cultures used:

M. S. C. 226, received from C. D. Sherbakoff, April 20, 1924, as No. 887g, from L. L. Harter's No. F-1;

M. S. C. 169, received from G. K. K. Link as No. 1592, originally from L. L. Harter's No. 1.

On malachite green, the growth is white, not broader than 2 cm. in the second step (1:200,000), stopping sharply after the third step (1:100,000), growth in the fourth step being either entirely suppressed or at most slight wefts pushed out here and there. No change in the color of the medium was detectable. The edge of the growth was typically even. The growth-form varied from submerged to cottony, villous, or sericeous. On crystal violet, the growth was white, extending practically through the standard series (1:4,444 or 1:4,000), restricted throughout (broadest 2 cm.) but persisting as a white mat about .5 cm. broad in steps seven to ten. The growth on crystal violet may be surrounded by a halo, especially in the stronger concentrations. The edge was even, except where sericeous wefts falling on the medium made the edge uneven. The growth-form was typically villous or sericeous. See Table 12.

On the standard medium without the dye, the growth was white, 4-5 cm. broad, circular, and the growth-form ranged from submerged with sericeous center to a woolly or villous growth.

Accessions 226 and 169 evidently were from the same original culture by Harter. These two subcultures in spite of a varied history in two labortories seem to respond in salient characters essentially alike.

Fusarium radicicola Wollenweber (Martiella section)

Cultures used:

M. S. C. 202, received from R. W. Goss as No. 37, originally from Shapavalov as Washington No. 212;

M. S. C. 203, received from R. W. Goss as No. 40, originally from McMillan as Washington No. 1400.

On malachite green medium, standard series, the mycelial mat was white, and growth was limited sharply to the first two steps. No change in the

medium occurred, the edge was even, except where the growth-form was sericeous and the matted wefts had fallen upon the medium. The growth-form was sericeous or in some cases cottony. On media containing crystal violet, the growth was white, restricted in breadth in comparison with growth on plain synthetic medium, in no case being more than 2 cm. broad. The growth persisted throughout the standard range, and through the extended range to 1:2,857, and continued as a trace through the 1:2,000 concentration. Strong decolorization occurred throughout, the edge was even and the growth-form cottony or villous usually. On synthetic agar containing copper sulphate, growth occurred in m/400 concentration, but not in any greater concentration. In concentration m/600 to m/1000 growth progressively became stronger almost equalling the plain agar in the high dilutions. The organism seems fairly tolerant of copper. On plain synthetic agar, the growth was white, 4 to 5 cm. broad, with an even edge; the growth-form was cottony or villous, Table 12.

Several other accessions, such as 157, received from G. K. K. Link as 1507 (Shapavalov 211), and 207, Goss 44 from L. L. Harter 3361, were not considered typical by C. D. Sherbakoff and have not given readings consistent with organisms taken as the type of this species. See Plate 4, F.

A, Group 5.

Fusarium euoxysporum Wollenweber (Elegans section)

Culture used:

M. S. C. 161, received from G. K. K. Link as No. 1633, originally from H. W. Wollenweber as No. 409.

On the standard malachite green series, *Fusarium euoxysporum*, as represented by this accession, produced a strong white growth, in many cases 3 to 4 cm. broad on the lowest malachite green concentration, 1:400,000, gradually diminishing in breadth of growth to about the middle of the range or slightly beyond. There was, typically, no change in the dye medium, the edge was even, and the growth-form was cottony. On crystal violet, the growth was white, persisting practically throughout the standard range. The growth on the crystal violet medium was only 2 cm. on the lowest concentration, 1:40,000, as contrasted with a 5 cm. breadth in the plain synthetic medium. No decolorization of the crystal violet occurred, the edge was even, and the growth-form cottony (11.001). On the brilliant green, standard series, growth was white, persisting through the range (1:22,222). The dye was strongly decolorized, the edge, even, and the growth-form cottony, Table 16. On plain synthetic agar, the growth was white, 5 cm. broad, circular in outline, and of cottony growth-form, (15.-01).

Fusarium alluviale Wr. and Reinking (Martiella section)

Culture used:

M. S. C. 294, received from C. L. Shear as No. 862, isolated from soil, Trujillo, Honduras.

On the malachite green standard series, this organism as represented by the single isolation available, produced a white growth extending to mid-range or beyond. The growth in many tests was 3-4 cm. broad in the 1:400,000 dilution, but was restricted in the greater concentrations. No

Table 16—Reactions of various Fusaria of 'A, Group 5' to a graded series of concentrations of malachite green, of brilliant green, of crystal violet, and of copper sulphate in synthetic agar.

Malachite Green Concentrations

Species	Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
F. euoxysporum	161	Apr. 27, 1929	—	13.001	—	12.001	—	11.001	—	tr	—	tr
F. alluviale	294	Apr. 14, 1929	—	—	11.003	—	0	—	11.001	—	0	0
F. moniliforme	229	Mar. 10, 1927	13.001	12 001	11.001	11.001	tr	tr	0	0	0	0
F. moniliforme	299	Apr. 27, 1929	—	13.008	—	11.001	—	0	—	0	—	0
F. malli	163	Mar. 10, 1927	12.001	12.001	11.001	11.001	11.001	0	0	0	0	0
F. malli	329	Apr. 27, 1929	—	13.001	—	0	—	0	—	0	—	0
F. solani	216	Apr. 21, 1927	14.201	14.201	11.201	0	0	0	0	0	0	0
F. solani	173	Apr. 21, 1927	13.201	11.201	tr	0	0	0	0	0	0	0
F. solani	313	Apr. 17, 1927	14.202	13.203	12.203	11.–06	0	0	0	0	0	0
F. solani	313	Apr. 25, 1928	—	12.213	—	11.203	—	0	—	0	—	0
F. solani var. minus	311	Apr. 21, 1927	15.201	14.201	14.201	14.201	tr	0	0	0	0	0

Brilliant Green Concentrations

Species	Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
F. euoxysporum	161	May 10, 1927	13.201	13.201	13 201	13.201	12 201	12 201	11 201	11 201	11 201	11.201
F. solani	216	July 3, 1927	14 001	13 005	13 005	12 001	tr	tr	tr	0	0	0
F. solani	173	July 3, 1927	14.001	13 001	13.001	12 001	tr	0	0	0	0	0
F. solani var. minus	311	July 3, 1927	14 000	13 006	13 006	12 005	tr	tr	0	0	0	0

Crystal Violet Concentrations, Standard Series

Species	Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
F. euoxysporum	161	Mar. 6, 1928	—	11 001	—	11 001	—	11 001	—	11 001	—	11 001
F. alluviale	294	Mar. 19, 1929	—	13 010	—	11 013	—	11 013	—	11 013	—	tr

Table 16—Continued.

Crystal Violet Concentrations, Extended Series

Species	Accession Number	Date of Test	1:20,000	1:10,000	1:6,666	1:5,000	1:4,000	1:3,333	1:2,857	1:2,500	1:2,222	1:2,000
F. moniliforme.....	229	Apr. 19, 1929....	12.001	11.001	11.001	11.001	11.001	12.001	11.001	11.001	11.001	11.001
F. moniliforme.....	299	Apr. 19, 1929....	12.008	11.001	11 001	11.001	11.001	11.001	11.001	tr	11.001	0
F. malli.....	163	May 9, 1929....	13 202	12.202	12.202	12.202	12.202	12 202	12.202	12.202	12.202	12.202
F. malli.....	329	May 9, 1929....	13 202	13.202	12.202	12.202	12.202	12.202	12.202	12.202	12.202	12.202
F. solani*.....	216	May 9, 1929....	14.201	11 201	11.201	11.201	11.201	11.201	11 201	11.201	11.201	11.201
F. solani*.....	173	May 9, 1929....	14.207	11.213	11.213	11.213	11 213	11.213	11.213	11.213	11.213	11.213
F. solani*.....	313	May 9, 1929....	14.206	12.213	12.213	12.213	12.213	12.213	12.213	12.213	12.213	12.213
F. solani var. minus.	311	May 9, 1929....	14.206	12.213	12.213	12.213	12.213	12.213	12.213	12.213	12.213	12.213

*In subsequent tests, some accessions grew to 1:1,666 concentration.

Copper Sulphate Concentrations

Species	Accession Number	Date of Test	m/1000	m/900	m/800	m/700	m/600	m/500	m/400	m/300	m/200	m/100
F. euoxysporum....	161	Jan. 10, 1929....	12 000	12 000	12 000	12 000	12.000	12.000	11.000	tr	0	0
F. malli.....	163	Jan. 24, 1929....	13 001	13 001	13 001	13 001	13 001	13 001	12.001	0	0	0
F. malli.....	329	Jan. 24, 1929....	14 001	14 001	14 001	13 001	13 001	13 001	12.001	11.001	0	0
F. solani	216	Jan. 24, 1929...	14 001	14.001	14 001	14 001	14 001	14 001	13.001	13.001	0	0
F. solani.....	173	Jan. 15, 1929....	22 000	22 000	22 000	22 000	22 001	22 001	12 001	11 001	0	0
F. solani.....	313	Jan. 15, 1929...	13.001	13.001	13.001	13.001	13 001	12 001	12.001	11.001	0	0

decolorization was produced, the edge of the colony was even, and the growth-form sericeous (11.003 or 11.001). On crystal violet, standard series, the mycelial growth was white, about 3 cm. broad in the 1:20,000 concentration, then less than 1 cm. (11.013), persisting through the entire range (1:4,000). On the extended series, slight growth persisted, "tr", through the 1:2,222 concentration. No decolorization was observed. The edge was irregular, and the growth-form was typically sericeous. In the lowest concentrations, the organism showed strong, revolute flutings on the mycelial mat. This feature may be more or less characteristic of the organism. On plain synthetic agar, the growth was strong and submerged, or often submerged with cottony fringe, atoll growth-form (14.007).

Fusarium moniliforme Sheld. (Liseola section)

Cultures used:

M. S. C. 229, received from C. D. Sherbakoff as No. 101, isolated by C. D. Sherbakoff from corn. Received April 20, 1924.

M. S. C. 299, received from C. L. Shear as No. 884, isolated from banana.

On synthetic medium containing malachite green, standard series, the growth was white and limited to the lower half of the series (i. e. to about 1:50,000). The growth in lowest concentrations was 2-3 cm. broad and very restricted in third or fourth step (.5 to 1 cm.). No change in medium color was noted. The edge was even and the growth-form, cottony or submerged with a cottony center. On the synthetic medium containing crystal violet, the growth occurred, although restricted in breadth, throughout the entire standard range, and practically through the extended range (1:2,000). The growth was white, about 2 cm. broad in the first step, quickly reduced to 1 cm. in higher concentrations, producing no change in the medium color; with even edge, and prevailingly cottony in growth-form, Table 16. On plain synthetic agar, the growth was white, 4-5 cm. broad, even edged, and cottony.

Fusarium malli Taub. (Martiella section)

Cultures used:

M. S. C. 163, received from G. K. K. Link, February 4, 1924, as No. 1660, originally from J. J. Taubenhaus who sent it to H. H. Whetzel, Cornell University;

M. S. C. 329, received from C. L. Shear as No. 936, originally from International Bureau of Fungous Cultures, Holland.

On synthetic media containing malachite green, standard series, the growth was white, moderately strong, (about 2½ to 3 cm. broad in the 1:400,000 concentration) and typically persisting through midrange. No change in the dye medium was observed, the edge was even and the growth-form typically cottony. On crystal violet, the growth was white, and persisted through the entire extended range (1:2,000), thus showing the great tolerance of the dye of the two accessions. The dye was decolorized by the fungus in all dilutions. The edge was even, and the growth-form villous throughout, Table 16.

This species, as represented by the two accessions used, differs only slightly from *Fusarium solani* and possibly should not be considered as other than

a strain of *solani*. In these tests, it has been slightly more sensitive to malachite green as measured by breadth of mat produced in the lower concentrations, and it has not decolorized the dye consistently.

Fusarium solani (Mart.) App. & Wr. (Martiella section)

Cultures used:

M. S. C. 216, received from C. D. Sherbakoff, April, 1924, as No. 711b;
M. S. C. 173, received from G. K. K. Link, February 4, 1924, as No. 1534, originally from Shapavalov's No. 213;
M. S. C. 313, received from C. L. Shear, as No. 906, under name *"F. radicicola";*
M. S. C. 311, (variety *minus*), received from C. L. Shear, December, 1924, as No. 902, isolated from soil, Honduras. Labelled "var. *minus*."

On synthetic agar containing malachite green, standard series, this organism produced in the lowest concentration, 1:400,000, a strong, white growth, almost equal to the plain agar without the dye. A strong growth also was produced in the second step, 1:200,000. The growth was restricted to about the lower third of the range, stopping at about 1:66,666 concentration. All Accessions strongly decolorized the dye. The edge, typically, was even. In Accessions 216 and 173, the growth-form was cottony; with Accession 313, villous or sericeous. Accession 311, *F. solani* var. *minus*, agreed fairly well with others of this group. On synthetic agar containing crystal violet, the growth was white and persisted throughout the entire extended range, measuring .5 to 1 cm. broad through the 1:2,000 concentration. The growth in the lowest concentration, 1:20,000, was strong, becoming gradually reduced in breadth. The dye was strongly decolorized throughout the series. The edge was even, except where the growth-form was sericeous and the wefts falling had produced an uneven edge. The growth-form varied, being cottony or sericeous. On the brilliant green medium, standard series, growth was restricted to lower half of the range, growth usually being checked between the fourth and fifth step. The growth in lowest concentration, 1:400,000, was almost the same breadth as that in plain agar. On synthetic agar containing copper sulphate the species, as represented by Accession 216, grew strongly (13.001) in m/300 concentration of the copper sulphate and in the higher dilutions, the restricting effect of the chemical almost entirely disappeared (i. e. reading for m/500 was 14.001). Other accessions were more sensitive. Accession 173 produced red color in the mycelium under the influence of copper sulphate. *Fusarium solani* seems rather tolerant of copper sulphate, and this may be of value in separation of *Fusarium solani* from some other species. On plain synthetic agar, the growth was strong, 4 to 5 cm. broad. The growth-form was cottony, or submerged with cottony center, (15.-01 or 15.-05). See Plate IV, F.

A, Group 6.

Fusarium martii App. & Wr. var. *viride* Sherb. (Martiella section)

Culture used:

M. S. C. 304, received from C. L. Shear as No. 892, isolated from citrus root.

On malachite green, extended series, growth, although restricted somewhat in lowest concentrations, persisted to a concentration of 1:11,111. The growth

was white, 2 cm. broad up to a concentration of 1:12,500. The malachite green was sharply decolorized throughout, the edge was even, and the growth-form cottony. On crystal violet, growth occurred through about three-quarters of the standard range; apparently, more sensitiveness was shown to this dye than to malachite green. The breadth of growth was 2 cm. in the lower concentrations, and then less than 1 cm. broad at 1:5,714. The growth was white, no decolorization occurred, the edge was uneven, and the growth-form sericeous, Table 17. On plain synthetic agar, the growth was white, 4 cm. broad, with even edge, and typically villous. (14.002).

Fusarium javanicum Koorders (Martiella section)

Culture used:

M. S. C. 298, received from C. L. Shear as No. 882, isolated from soil, Honduras.

On synthetic agar containing malachite green, the growth was white and moderately strong (3 cm. broad in second step), persisting through 1:25,000 concentration. Malachite green was strongly decolorized, the edge was uneven, and the growth-form sericeous. This organism showed greatest tolerance of crystal violet of any tested, growing strongly in all of the standard series, the extended series, and persisting in still higher concentrations up to a 1:1,250 concentration of the dye. The growth was white, and the dye was sharply decolorized. The edge was ramose, and the growth-form sericeous. On plain synthetic agar, the growth was broad (4 cm.) with even edge and of villous growth-form.

B. 1′

Fusarium tricinctum (Corda.) Sacc. (Roseum section)

Culture used:

M. S. C. 254, received from H. W. Wollenweber, July, 1924, as No. 1528.

On malachite green, standard series, no growth in any concentration. In a dilute series, weak growth (less than .5 cm.) occurred in a concentration of 1:666,666 of dye, (11.000). No change in the dye could be discerned, the growth was even and completely submerged. The organism seemed very sensitive also to crystal violet, no measurable growth occurring in any dilution of the standard series, Table 18. Growth on the plain synthetic agar was red, 2 cm. broad, irregular in outline, and submerged.

B. 2′

Fusarium paspalicola P. Henning (Discolor section)

Culture used:

M. S. C. 273, received from H. W. Wollenweber as No. 1570.

On malachite green, standard series, no measurable growth occurred on any dilution (lowest concentration 1:400,000). On a series still more dilute, growth occurred only at the dilutions 1:2,000,000 and 1:666,666 and this was less than 1 cm. broad, with a frondose edge, and cottony growth-form, (21.-21), Table 18. No growth occurred in the standard series of crystal violet dilutions. On plain synthetic agar, the color of the mycelium was red.

Table 17—Reaction of *Fusarium martii* var. *viride* and *Fusarium javanicum*, 'A, Group 6', to various concentrations of malachite green and of crystal violet in a synthetic agar.

Malachite Green Concentrations, Extended Series

Species	Accession Number	Date of Test	1:100,000	1:50,000	1:33,333	1:25,000	1:20,000	1:16,666	1:14,285	1:12,500	1:11,111	1:10,000
F. martii var. viride..	304	Apr. 14, 1929....	12.201	12.201	12.201†	12.201†	12.201	12.201	0	0	11.201	0
F. javanicum.....	298	Apr. 14, 1929....	13.203	11 213	12 213	12 213	0	0	0	0	0	0

Crystal Violet Concentrations (Standard Series)

Species	Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
F. martii var. viride	304	June 20, 1925....	12.013	12.013	12.013	11.013	11.011	11.013	11.013	tr	tr	0

Crystal Violet Concentrations (4× Series)

Species	Accession Number	Date of Test	1:10,000	1:5,000	1:3,333	1:2,500	1:2,000	1:1,666	1:1,428	1:1,250	1:1,111	1:1,000
F. javanicum.....	298	Apr. 29, 1929....	12.213	12.213	12.213	12.213	11.213	11.213	11.213	11.213	0	tr

Table 18—Reactions of various Fusaria of "B" grouping (mycelium red) to a series of concentrations of malachite green and of crystal violet in a synthetic agar and on synthetic agar without dye.

Malachite Green Concentrations

Species	Accession Number	Date of Test	1:400,000	1:200,000	1:100,000	1:66,666	1:50,000	1:40,000	1:33,333	1:28,571	1:25,000	1:22,222
F. tricinctum	254	Apr. 20, 1929	0 tr	0	0	0	0	0	0	0	0	0
F. paspalicola	273	Apr. 20, 1929	tr	0	0	0	0	0	0	0	0	0
F. culmorum	218	Apr. 14, 1929	23.000	22.000	22.000	0	—	0	—	0	—	0
F. culmorum var. leteius	219	Apr. 14, 1929	24.021	24.021	23.021	—	22.021	—	—	—	21.021	22.021
F. decemcellulare	293	Apr. 14, 1929	22.001	—	21.001	—	21.001	—	0	—	0	—

Malachite Green Concentrations, Approximate Growth-Range Series

Species	Accession Number	Date of Test	1:2,000,000	1:666,666	1:400,000	1:285,714	1:222,222	1:200,000	1:66,666	1:40,000	1:28,571	1:22,222
F. paspalicola	273	Mar. 29, 1929	21.–21	tr	0	0	0	0	0	0	0	0

Crystal Violet Concentrations, Standard Series

Species	Accession Number	Date of Test	1:40,000	1:20,000	1:13,333	1:10,000	1:8,000	1:6,666	1:5,714	1:5,000	1:4,444	1:4,000
F. tricinctum	254	May 3, 1929	—	tr	—	tr	—	tr	—	0	—	0
F. paspalicola	273	June 28, 1925	0	0	0	0	0	0	0	0	0	0
F. culmorum	218	Apr. 24, 1929	—	tr	—	0	—	0	—	0	—	0

Table 18—Continued.

Crystal Violet Concentrations, Extended Series

Species	Accession Number	Date of Test	1:20,000	1:10,000	1:6,666	1:5,000	1:4,000	1:3,333	1:2,857	1:2,500	1:2,222	1:2,000
F. culmorum var. leteius	219	Mar. 19, 1929....	23 021	22 021	21.021	21.021	21.021	21.001	21.001	21.001	21.001	21.001
F. decemcellulare..	293	Mar. 19, 1929....	22 011	21.011	21 011	21.011	21.011	21.011	21.011	21.011	21.011	tr

Synthetic Agar without Dye

F. tricinctum.....	254	Apr. 20, 1929....	22.010									
F. paspalicola.. ..	273	Apr. 20, 1929....	24.001									
F. culmorum	218	Apr. 14, 1929....	22.001									
F. culmorum var. leteius.	219	Apr 14, 1929....	24.021									
F. decemcellulare..	293	Apr. 14, 1929....	24.001									

The growth was 4 cm. broad, with even edge, and typically cottony, (24.001). See Plate I.

B. 4′

Fusarium culmorum (W. G. Smith) Sacc. (Discolor section)

Culture used:

M. S. C. 218, received from C. D. Sherbakoff, April 20, 1924, as No. 803e, (determined by Miss J. P. Rose).

On malachite green, the red-colored growth was limited to the lowest quarter of the range, 2 cm. broad in the 1:100,000 concentration. No decolorization was produced. The edge was even, and the mycelium completely submerged, (22.000). On crystal violet, no measurable growth occurred in the standard series. On plain synthetic agar, the growth was red, circular, submerged or cottony, Table 18.

B. 5′

Fusarium culmorum Sacc. var. *leteius* Sherb. (Discolor section)

Culture used:

M. S. C. 219, received from C. D. Sherbakoff, April 20, 1924, as No. 803i, (determined by Miss J. P. Rose).

On the synthetic medium containing malachite green, standard series, growth was very strong in the lowest concentrations and persisted through the entire range. The mycelium color was red. No decolorization of the dye agar occurred. The edge of the mat was very frondose, and the growth-form was cottony. On the synthetic medium containing crystal violet, extended series, the growth occurred through dye concentration of 1:2,000. The color of the mycelium was red throughout, the breadth in lowest concentration was 3 cm., 2 cm. at mid-range, and then about 1 cm. broad through the stronger concentrations. No decolorization was noted. The edge of the mat was usually uneven, and the growth-form, cottony. On plain synthetic media the growth was strong, red, frondose in outline, cottony. (26.021; 24.021.)

The tolerance of malachite green and crystal violet shown by this organism is in such strong contrast to the behavior of *Fusarium culmorum* as to suggest the desirability of further study of this variety.

B. 5′

Fusarium decemcellulare Brick. (Spicarioides section)

Culture used:

M. S. C. 293, received from C. L. Shear, as No. 875, isolated from cacao twigs affected with die-back, Panama.

On synthetic agar containing malachite green, standard series, this organism grew through the 1:50,000 concentration. The color of the mycelium was red, the growth moderately strong, the medium not decolorized in all dilutions. The edge of the colony was even, and the growth-form cottony. On synthetic agar containing crystal violet, extended series, the growth continued throughout the standard range and through a dye concentration of 1:2,000. The growth was restricted (1 cm. broad), and no decolorization

occurred, the edge of the mat was uneven, and the growth-form was cottony. On plain synthetic agar, the growth was only moderately strong (3 to 4 cm.), frequently irregular in outline, and cottony in growth-form, Table 18.

GENERAL DISCUSSION

In the record of readings and the provisional keys based upon them, an attempt has been made to show that the species of Fusarium, as represented by the available authentic cultures, have given readings, which, if variability properly attributable to the technic is taken into account, seem consistent. Any interpretation of such reactions as are reported here should take into account that individual lots of aniline dyes may vary slightly in dye content and other factors also are operative which militate against mathematical exactness in any two sets of responses. The wording of the keys indicates that the growth limit is "about" at such a concentration and the growth record has been taken in such a way as to minimize slight differences. The decolorization reaction to various dyes is probably precise and except where the medium is of such low or great concentration as to obscure the reading, this reaction can probably be used safely. The form of the colony is definite with those organisms which are remarkably frondose in outline, such as *Fusarium culmorum* var. *leteius* and *F. semitectum*. In many other cases, the irregularity of the edge has arisen from the falling of sericeous threads. As has been discussed, the type of aerial growth, while the most striking thing noticed when the plates are examined, has been rather variable and the value of this criterion is somewhat doubtful.

It is the authors' opinion, based upon consideration of the representative readings reported and the many others of confirmatory nature which have been obtained in this work, that the broad limits as indicated by the main divisions in the keys, and the major subdivisions in the keys indicate the reactions to be expected from an organism which, in the hands of a specialist skilled in diagnosis of Fusarium, would be assigned to the species indicated. For example, it is confidently expected that true *F. cumartii* or *F. cubense* cultures would be extremely sensitive to malachite green and to crystal violet, and that *F. niveum*, *F. lycopersici*, and *F. vasinfectum*, in their sensitiveness to malachite green, would have an intermediate position between the extremely sensitive organisms named and *F. oxysporum*, which is moderately sensitive. Because of its tolerance of malachite green, *F. solani* and its allies can be expected to be sharply marked off from those mentioned, and the extreme tolerance of this species of crystal violet as well, make easy its differentiation from such forms as *F. cumartii*—a separation very difficult to make on morphological grounds. Many of the species, because of listed peculiarities in form or growth on standard media, can be quickly placed in the proper section now recognized by the specialists. For species differentiation within these groups, as well as to pick out the outstanding and well-marked reactors, the dye method can prove most useful.

Probably the simplest method of utilizing the differentiating powers of the dye media would be to grow a known culture in the same plate with the suspect. Such direct comparison would quickly reveal similarities or differences. It is proposed that reliance be placed on such findings in deciding the likeness and unlikeness between organisms.

An evaluation of the limits of dye method for such diagnostic purposes as is proposed can be made by consideration of the accessions ob-

tained from different laboratories which trace back to the same original isolation. It has been possible to compare these under a variety of conditions, after some years of maintenance in different laboratories. Such opportunity for comparison is afforded by the *F. oxysporum* accessions 211, 204, and 55, which trace back to an isolation made by Department of Agriculture workers and distributed about 1915 as "Washington 3395." Representative reactions obtained with these three accessions are given in Table 15 for malachite green, crystal violet, and brilliant green. These reactions show consistent agreement. The deviations shown are a measure of the degree of reliance one can attach to an individual reading.

F. batatatis accessions, 226 and 169, also apparently trace back to an isolation made by L. L. Harter and distributed by him as No. 1. The readings for these two accessions agree excellently in their salient features and give another check on the method. (Table 12.)

It will be noted that in this work, the culture, after the first plating to furnish inoculum from what was apparently a single spore, was maintained as a stock culture on standard laboratory media, such as potato dextrose agar, and this stock culture was used without any preliminary culturing as a direct source for inoculating the synthetic media, on which the inoculum threads were laid. No attention was paid to the sporulation being produced since the purpose of this work was to seek a practical method which involved little delay. Such closely tallying responses in dye sensitiveness as have been obtained are in sharp contrast to the reported experiences where cultures which have been maintained for a long time in the laboratory have been used in morphological studies or even in culture tests on ordinary laboratory media.

It is believed the showing made in the many repetitions of the test with those accessions which trace back to a common source, and the close duplication of readings which has resulted in the repeated tests of the various accessions of carefully identified species under observation, warrant the conclusion that there is enough constancy in the reactions to the aniline dyes to make them usable for diagnostic purposes if a suitable technic is employed.

Opposite ground to Leonian's published conclusions (9) with respect to the species of the genus Fusarium is taken in drawing this conclusion. It should be noted, however, that Leonian (10) in his study of the reactions of various species and strains of Phytophthora arrived at the conclusion that the reactions of the Phytophthora to malachite green would offer convenient means of distributing the many existing strains. The genus Fusarium in his work did not give the uniformity of reaction found with Phytophthora. Leonian determined the reactions of 95 species and varieties of Fusarium to various chemicals, including malachite green. Using a synthetic solution, he added malachite green to give 1:500,000, 1:200,000, 1:100,000, 1:50,000, 1:25,000 and 1:10,000 concentrations. This solution was inoculated with discs of agar and mycelium 5 mm. in diameter cut from the outer edge of growth or, in the case of old plates, at the border. Leonian used, in large part, for his work Fusaria obtained from Dr. C. L. Shear and from various sources which supplied the cultures used in this work. Since no culture numbers were retained, it is impossible to determine, in the great majority of cases, exactly what accession number used in the present work duplicates the culture which he used. Leonian arrived at diametrically opposite conclusions from the one drawn from this work. In his hands the Fusaria "dissociated" and his record of reactions obtained with malachite green, for example,

is a medley of disconsonant readings which in part is responsible for his expressed opinion "the only constant thing in Fusaria is their inconstancy."

Respecting malachite green reactions he remarks: "Generally speaking, however, there is more similarity of reaction toward malachite green than there is of dissimilarity; as even a casual glance at the foregoing table will reveal. Nevertheless, the same lack of uniformity which has characterized the reactions of Fusaria toward other environmental factors is to be noted in the following graphical matter" . . . (Diagramatic table follows).

The results reported in this paper, on the contrary, have not revealed such a state of inconstancy in repeated tests, and the writers would incline to attribute the erratic behavior of the Fusaria which Leonian reports to variations of conditions presented in the various tests, or to comparisons made between improperly identified cultures. The conclusions drawn from the work here presented are in accord with Sherbakoff's frank preface (9) of Leonian's work and, on the basis of these findings, the authors approve fully of Sherbakoff's statement: "The exceptions to the uniform response, the so-called erratic or inconsistent behavior as observed in all of the tests employed herein, will probably be found, after a thorough revision of the facts involved, either conditional or showing true differences in the organisms concerned."

Sectoring was not found in these platings. It is of course possible that they were overlooked. Acting on the assumption that they were overlooked the many photographs taken as records in this work were reviewed and in only one photograph has anything approaching a sector appeared. It is true that others have reported certain media as conducive to sectoring, and the medium used exclusively in this work and the period (10 days) may have not been favorable enough for this to occur.

With many publications showing that fungi, like other living things, are variable, and not a group apart in which all outgrowths are cast after an unvarying pattern, one perhaps should safeguard his statements with reference to sectoring, saltations, mutations, or variants as having been made without intent to deny that deviations from normal do occur, perhaps more frequently than chance observation would indicate. The true nature of these deviations is not as yet certain. It would seem that if these variants in Fusarium were occurring with the frequency reported for this genus (13), or for the other genera, and if these variations had significance so far as the species entity, as determined by the dye reactions, is concerned, they would have made themselves manifest some place in these tests or in the stock cultures, and any great approach to constancy of reaction over a long period of time would have been precluded.

Brown (13) and Mitter (14) have questioned the validity of recent work of classification of Fusaria since, in their opinion, the variants derived from a single spore have so transcended the species limits, as given by taxonomists of the group, that the variants of a single form studied apparently could be classified in different species.

The authors of this bulletin are not in position to judge directly the findings of Brown and Mitter since, in this work there was no study of spore form or other criteria used in classification, but attention is called to the striking parallelism in the independent classification of the cultures by a Fusarium specialist and the dye reactions. If the assignment, as done by Sherbakoff, of members of the Elegans

section into species, were unreliable, to take only this section as an example, and resulted in divergent forms such as *F. cubense,* and *F. oxysporum,* and others of the Elegans section being not "satisfactorily divided up," as Hansford (15) states is the true situation when present methods of classification are attempted, it would seem that contradictions in the dye responses would have been inevitable.

In the two cases (*F. oxysporum* and *F. batatatis*) where accessions which had a varied culture-history but which in each case arose from a single source culture were available for morphological and cultural study, Dr. C. D. Sherbakoff was able to confirm the early species-diagnosis by his examinations. The results of the authors' readings were accepted as proof that the respective accessions for each species behaved essentially alike and conformed to the readings of other properly identified accessions. Dissociation, saltation, or variation in these accessions over a long period of culture had not led to marked departure in reaction to dye from the norm of the species. It will be noted, also, that in response to the dyes, the recognized varieties of several species usually took a fairly close position to the species itself. The original grouping into species and varieties had been made on the standard bases used in diagnosing the species of the genus. As with growth, color reaction, and spore size, there seems to be a mean value for the response to the dyes around which the reactions of related forms cluster. So far as the authors' experience extends, it would seem that the present accepted subdivisions of the genus Fusarium into species represent recognizable entities and the agreement in response to dyes within these entities is another indication of the likeness which exists between the members assigned to a particular species.

Radical divergence in readings obtained in many repetitions of tests with the same culture was not found. If radical deviation occurs, variation in the conditions presented, error in the manipulations, or the occurrence of contamination should, in the writers' opinion, first be suspected. Suggestion that significant change in the genetic nature of the culture has taken place should be advanced only when the chance of such extraneous circumstance is eliminated.

That such genetic changes do and have taken place, perhaps sparingly with some forms, in the genus Fusarium is evidenced by the very complexity of the genus at present. That different physiological strains exist among fungi is now common knowledge. In this regard, fungi behave like other living organisms. The significance of these physiological and otherwise aberrant forms in relation to a concept of species of fungi is yet to be determined. The relative rank in a classification scheme, that these entities shall have does not seem the significant question. The question is rather if any of the present groupings made on morphological grounds represent entities permanent enough to warrant a distinguishing designation. Since the forms under observation seem to have been fairly constant over a long period of observation, we are therefore conservative enough to want to use present species designations for the genus until more evidence on the mutability of the species which would render definite designations futile is furnished.

SUMMARY

The Fusarium species are commonly recognized as difficult if not impossible for the pathologist who is not a specialist in the group to determine. As an auxiliary means of diagnosis, methods are outlined by which the laboratory worker can use the results obtained by growing his isolations upon culture media containing growth-inhibiting substances either for direct comparison of a suspect with a known culture or, so far as this list covers the species and is representative, to determine the species from the results obtained in this work.

It was found that many of the species and varieties of Fusarium tested showed such differences in color of mycelium, in breadth of growth, in ability to change the color of a dye, or in form or type of growth if grown on a synthetic medium containing an aniline dye such as malachite green or crystal violet as to permit sure differentiation. In this work, the triphenylmethane dyes were most useful but acriflavine and copper sulphate were also found to have possibilities.

The methods employed consisted essentially of growing, from a uniform mass of inoculum, the various species and varieties of Fusarium upon a synthetic medium containing the chemical presented in a graded series of concentrations. Petri dish cultures kept at constant temperatures under humid conditions in the dark were used.

Fifty-four species and varieties represented by 104 accessions whose trueness to type was checked by Dr. C. D. Sherbakoff were tested repeatedly by the methods outlined and the results obtained are catalogued. The variations in responses to the different aniline dyes have permitted the grouping of the species tested according to sensitiveness to or tolerance of malachite green, with reactions to crystal violet and other dyes being used for further separations. For convenience, a provisional "key" based on these responses to the dyes is given. The records obtained are recorded by means of a simple numerical scheme.

It was found by many repetitions of the tests over a period of several years that approximately the same results were obtained each time. Typical readings are given and the slight variations shown are believed assignable to variations inherent in the technic. In the case of two species, several subcultures from the original parent cultures were available for comparison. These subcultures in spite of a varied history of transfer in different laboratories still gave closely tallying responses indicating the apparent independence of the dye responses to the previous growth condition of the culture. The closeness of the physiological responses, coupled with the identity in morphology of these subcultures indicates that during a period of 10-15 years in artificial culture no significant change had occurred. The present system of classification of Fusaria has sought to separate out recognizable entities. Where several accessions of the same species of Fusarium were available, these properly identified cultures reacted with the degree of constancy to be expected.

Plate I.

A. *Fusarium eumartii,* accessions 200 and 215, and accessions 205 and 206 (considered atypical), growing on synthetic agar without dye. (Photographed April 29, 1928; period, 10 days.)

B. Same, on synthetic agar containing crystal violet, 1:20,000. (Photographed April 29, 1928; period, 10 days.)

C. *Fusarium eumartii,* accessions 200 and 215, accession 205 (atypical of *eumartii*) and accession 207 (received under label *"radicicola"*) growing on synthetic agar containing malachite green, 1:2,000,000. (Photographed May 1, 1929; period, 10 days.)

D. E. F. *Fusarium paspalicola,* (accession 273), *F. cubense* (227), *F. ensiforme* (296), and *F. chlamydosporum* (276).

D. Growing on synthetic agar without dye.

E. Growing on synthetic agar containing malachite green, 1:666,666.

F. Growing on synthetic agar containing malachite green, 1:285,714.

(D, E, F, photographed May 1, 1929; period 10 days.)

Plate II.

A-C. *Fusarium niveum.*

Accessions 418, 421, and 430 growing on synthetic agar of the following constitution:

A. Containing crystal violet, 1:20,000;

B. Containing crystal violet, 1:10,000;

C. Containing crystal violet, 1:4,000.

D-F. *Fusarium orthoceras* and varieties.

Accessions 158, 159, 188, 258, and 277 growing on synthetic agar containing dyes as follows:

D. Malachite green, 1:200,000;

E. Crystal violet, 1:10,000;

F. Crystal violet, 1:4,000.

(A, B and C photographed May 3, 1929; D, April 17, 1927; E, and F, Mar. 26, 1928; period 10 days.)

Plate III.

Fusarium lycopersici (accessions 153, 170, 210, 259, 434, 437 and 438) growing on synthetic agar of following constitution:

A. Without dye;

B. Containing acriflavine, 1:5,000;

C. Containing malachite green, 1:400,000;

D. Containing malachite green, 1:200,000;

E. Containing crystal violet, 1:6,666;

F. Containing crystal violet, 1:5,000.

(A, B, and D, photographed March 25, 1928; C and E, March 17, 1927; F, May 3, 1929; period, 10 days.)

Plate IV.

A-E. *Fusarium vasinfectum* (accessions 176, 253, 334, 420, 422, 427, 431, 432, 433, 435, and 439), growing on synthetic agar of following constitution:

A. Without dye;
B. Containing malachite green, 1:200,000;
C. Containing crystal violet, 1:20,000;
D. Containing crystal violet, 1:10,000;
E. Containing crystal violet, 1:6,666.

F. *Fusarium solani* (accession 313) and *F. radicicola* (accessions 157, 207, and 202), growing on synthetic agar containing malachite green, 1:200,000.

(A and B photographed April 29, 1928; C-E, May 3, 1929; F, April 17, 1927; period, 10 days.)

Plate V.

A-D. *Fusarium conglutinans* (accessions 156, 165 and 220) growing on synthetic agar of the following constitution:
A. Without dye;
B. Containing malachite green, 1:200,000;
C. Containing crystal violet, 1:20,000;
D. Containing acriflavine, 1:5,000.

E-F. *Fusarium conglutinans* (accessions 165 and 156), *F. neoceras* (accession 308), and *F. ossicolum* (accession 307) growing on synthetic agar of the following constitution:
E. Containing malachite green, 1:666,666;
F. Containing malachite green, 1:222,222.

(A, B, C, D, photographed April 29, 1928; E and F, April 20, 1929; period, 10 days.)

Plate VI.

A-D. *Fusarium hyperoxysporum* (accessions 162, 225, 270 and 332) on test synthetic agars as follows:
A. Without dye. (Photographed March 7, 1928; period 11 days.)
B. Synthetic agar containing malachite green, 1:400,000. (Photographed April 6, 1927; period 13 days.)
C. Synthetic agar containing malachite green 1:200,000. (Photographed March 7, 1928; period 11 days.)
D. Synthetic agar containing crystal violet, 1:20,000. (Photographed March 7, 1928; period 11 days.)

E-F. *Fusarium oxysporum* (accessions 204, 208, 209, 211, 212, 309, 310 and 321) on synthetic agar containing brilliant green, 1:50,000. (Photographed May 17, 1927; period 13 days.)

Plate VII.

Fusarium oxysporum.

Various accessions (55, 160, 161, 185, 204, 208, 209, 211, 212, 309, 310, and 321) growing on synthetic agar of the following constitution:
A, B, C. Containing malachite green, 1:400,000;
D. Containing malachite green, 1:200,000;
E. Containing crystal violet, 1:20,000;
F. Containing crystal violet, 1:10,000.

(A, B, and C, photographed April 6, 1927; period 13 days; D, March 7, 1928, period, 11 days; E and F, May 6, 1929, period 11 days.)

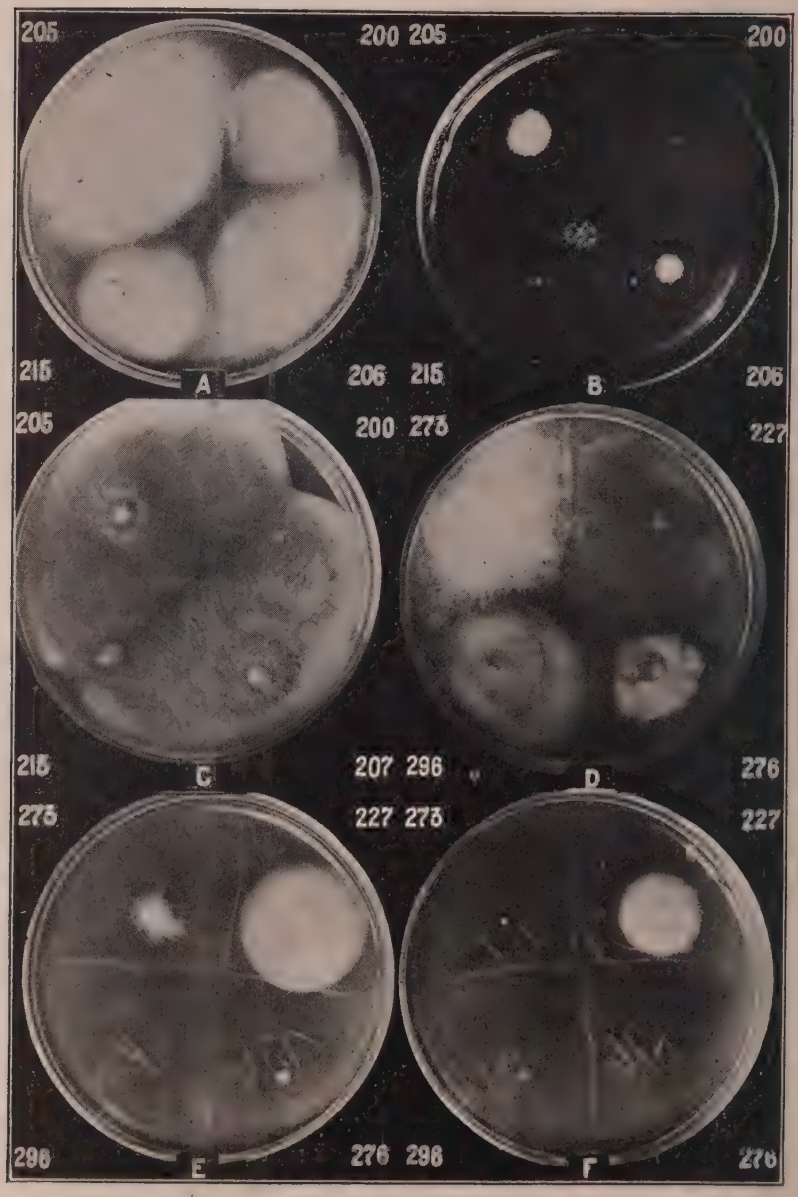

Plate I. *Fusarium spp.*

A. Without dye.
B. Crystal violet, 1:20,000.
C. Malachite green, 1:2,000,000.
D. Without dye.
E. Malachite green, 1:666,666.
F. Malachite green, 1:285,714.

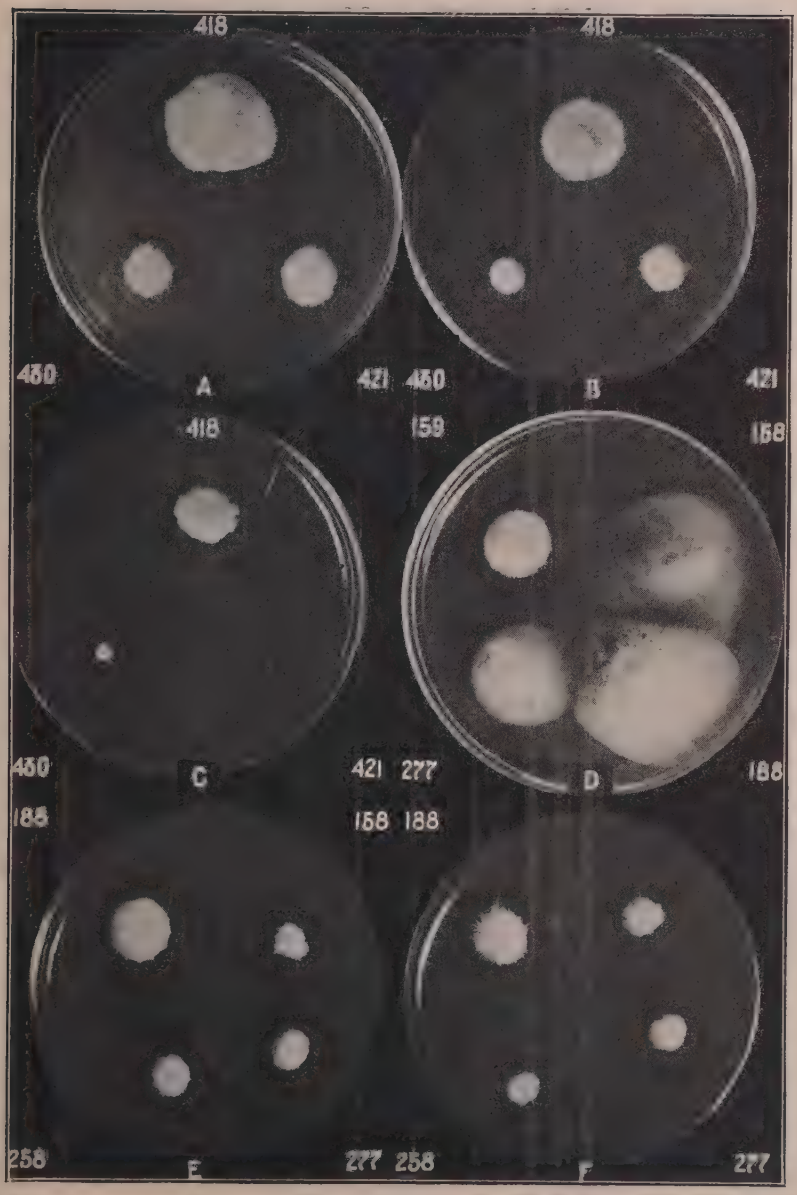

Plate II. *Fusarium niveum* and *F. orthoceras.*

A. Crystal violet, 1:20,000.
B. Crystal violet, 1:10,000.
C. Crystal violet, 1:4,000.
D. Malachite green, 1:200,000.
E. Crystal violet, 1:10,000.
F. Crystal violet, 1:4,000.

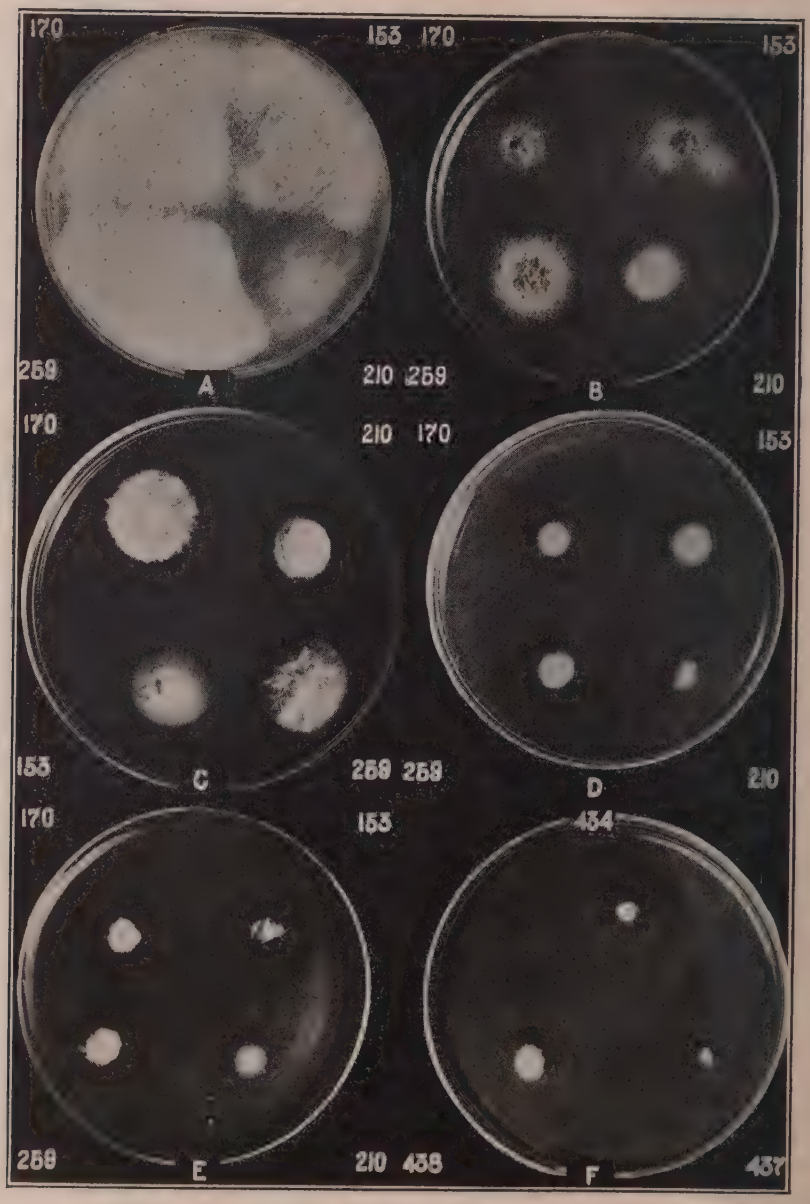

Plate III. *Fusarium lycopersici.*

A. Without dye.
B. Acriflavine, 1:5,000.
C. Malachite green, 1:400,000.
D. Malachite green, 1:200,000.
E. Crystal violet, 1:6,666.
F. Crystal violet, 1:5,000.

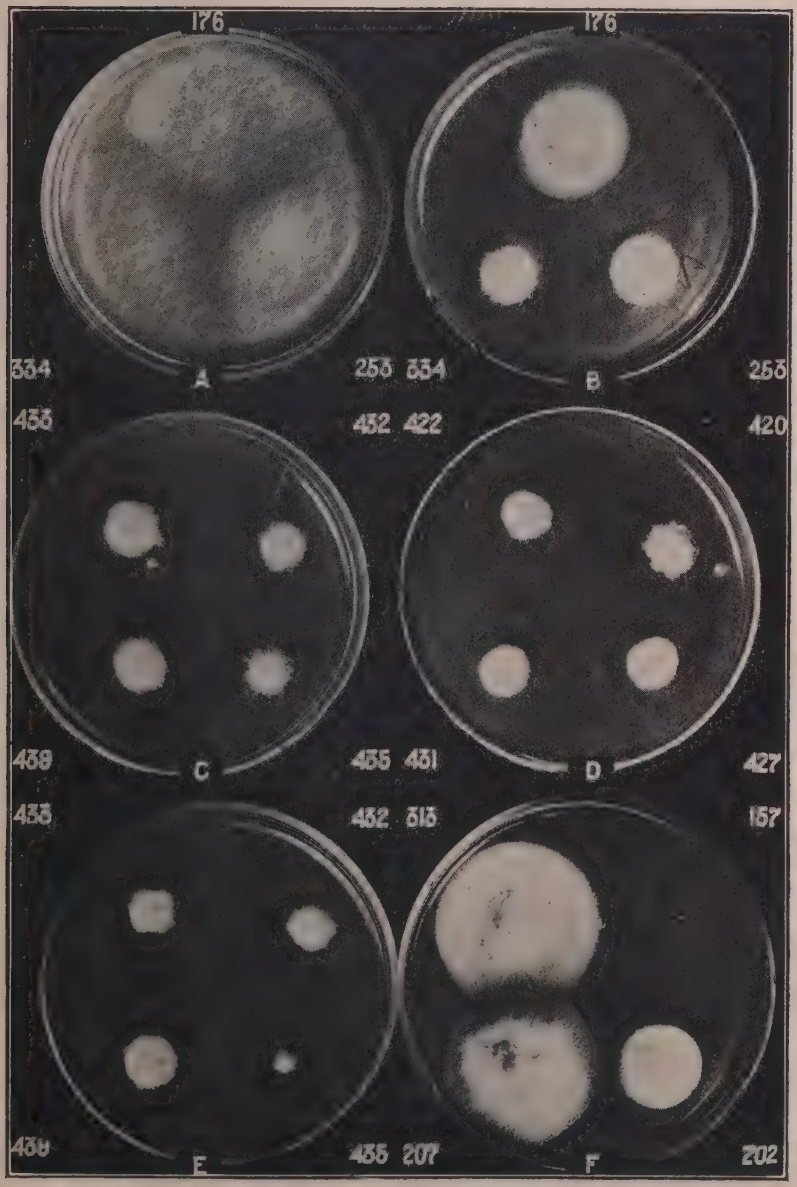

Plate IV. *Fusarium vasinfectum* (A.–E.) and *F. solani* and *F. radicicola* (F.).

A. Without dye.
B. Malachite green, 1:200,000.
C. Crystal violet, 1:20,000.
D. Crystal violet, 1:10,000.
E. Crystal violet, 1:6,666.
F. Malachite green, 1:200,000.

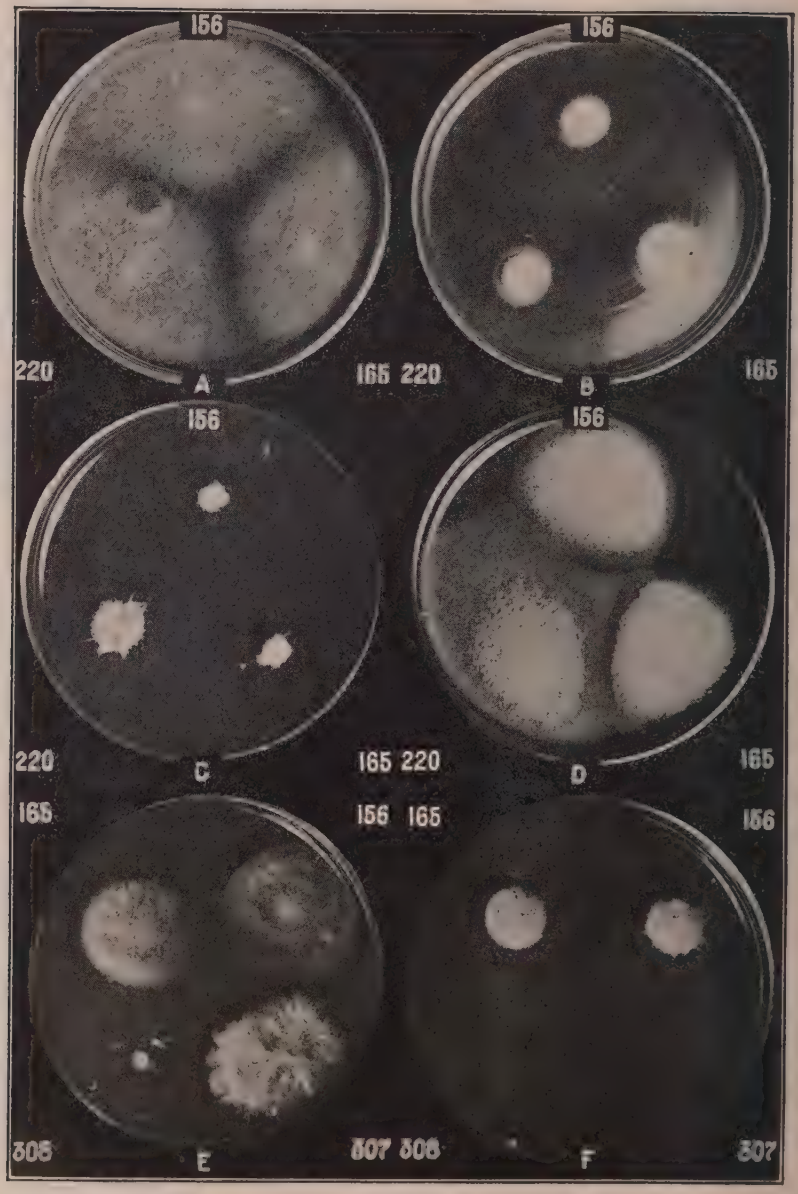

Plate V. *Fusarium conglutinans* (A.–D.); *F. conglutinans*, *F. neocerae* and *F. ossicolum* (E.–F.).

A. Without dye.
B. Malachite green, 1:200,000.
C. Crystal violet, 1:20,000.
D. Acriflavine, 1:5,000.
E. Malachite green, 1:666,666.
F. Malachite green, 1:222,222.

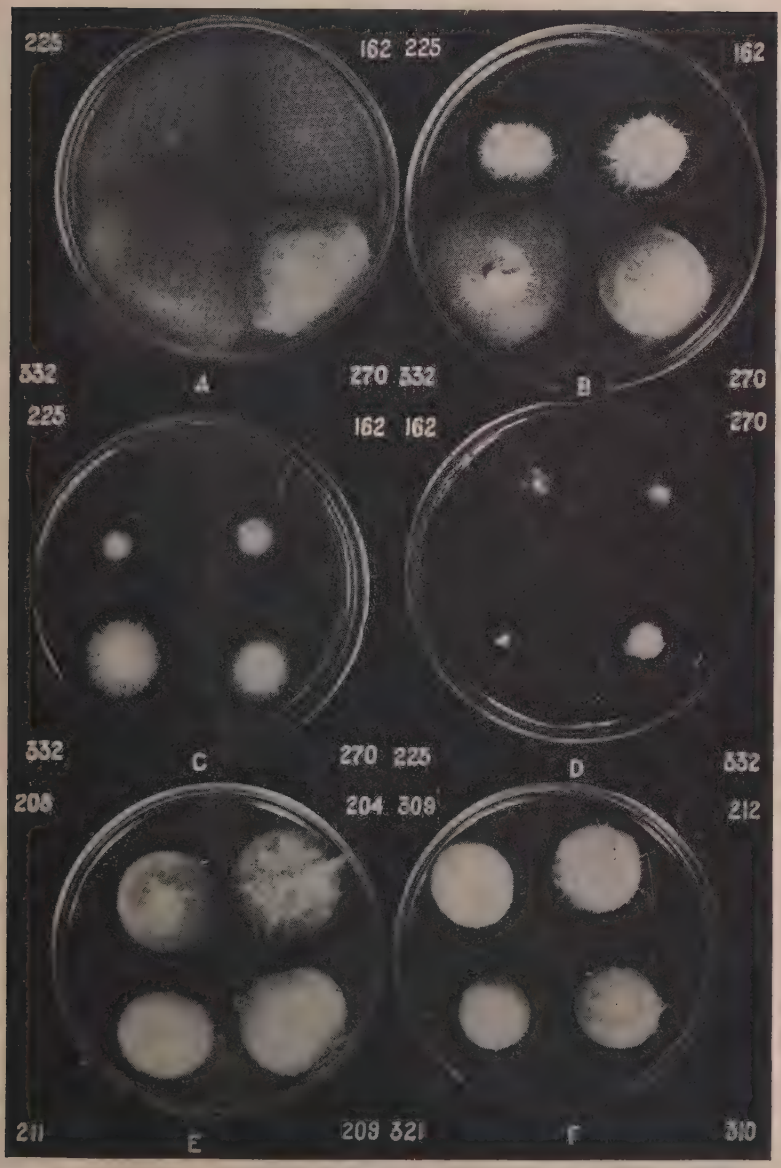

Plate VI. *Fusarium hyperoxysporum* (A.–D.) and *F. oxysporum* (E.–F.).

A. Without dye.
B. Malachite green, 1:400,000.
C. Malachite green, 1:200,000.
D. Crystal violet, 1:20,000.
E. Brilliant green, 1:50,000.
F. Brilliant green, 1:50,000.

Plate VII. *Fusarium oxysporum.*

A. Malachite green, 1:400,000.
B. Malachite green, 1:400,000.
C. Malachite green, 1:400,000.
D. Malachite green, 1:200,000.
E. Crystal violet, 1:20,000.
F. Crystal violet, 1:10,000.

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